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Effects of Inhalation of Cadmium on the Rat Olfactory System: Behavior and Morphology

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SUN, T.-J., M. L. MILLER AND L. HASTINGS. *Effects of inhalation of cadmium on the rat olfactory system: Behavior and morphology.* NEUROTOXICOL TERATOL **18**(1) 89–98, 1996.—To investigate the effects of cadmium on olfaction, two separate studies were conducted in which male adult rats were exposed to CdO, via inhalation, for 5 h per day, 5 days a week for 20 weeks. Target exposure values of 250 and 500 $\mu g/m^3$ were measured at 200 and 325 $\mu g/m^3$ for the low concentration in two experiments, and 550 and 660 $\mu g/m^3$ for the high concentration. Prior to exposure, olfactory thresholds were obtained using a conditioned suppression technique. After 20 weeks of cadmium exposure, there was no evidence of anosmia in any of the rats nor were there any significant changes observed in olfactory thresholds. Although olfaction was not impaired, cadmium levels in the olfactory bulbs of exposed rats were significantly elevated compared to controls. Cardiac and respiratory histopathology were observed at all exposure levels, but there was no evidence of nasal pathology related to exposure to cadmium. Failure of cadmium to produce olfactory dysfunction may be due to the protective effects of metallothionein and/or to the highly resilient nature of the rodent olfactory system.

Cadmium Olfaction Neurotoxicity Conditioned suppression Inhalation

A NUMBER of clinical studies of workers chronically exposed to cadmium suggest that such exposure may result in olfactory deficits, including anosmia. Friberg (17) reported that 37% of workers in a cadmium battery factory showed impaired olfactory function after about 20 years of exposure. Postmortem examination of a cadmium-exposed worker showed atrophy of the nasal mucosa and intensive yellow staining of the olfactory bulbs (4). In a reasonably well-controlled study, alkaline battery workers reported significantly more anosmia and performed significantly more poorly in the phenol smelling test than age-matched controls (1). The findings of a positive correlation of anosmia with proteinuria in this study further suggested that cadmium might be the offending agent. In a group of battery workers, olfactory damage was found in over 50% of workers exposed to cadmium for 10–29 years, and damage in 91% exposed for more than 30 years (33). Smelter workers exposed to cadmium fumes for varying periods of time at a zinc refinery experienced decreased olfactory acuity (43). In another group of cadmium smelter workers, 28% developed anosmia after being exposed to airborne cadmium oxide (0.004–0.187 mg/m^3) for more than 5 years. Finally, factory workers exposed to cadmium fumes from a brazing operation performed more poorly than control subjects on a standardized smell function test (36). Most of the cited studies involved cadmium oxide dust, but the last three also involved exposure to cadmium fumes. Although anosmia is less often reported to be associated with exposure to cadmium fumes (46), detailed information regarding exposure conditions is usually very sparse or absent in these studies. Nevertheless, there exists a number of human studies implicating some form of cadmium exposure with dysfunction of the olfactory system.

In animal studies, chronic exposure to cadmium in adult rats has been found to cause a significant increase in the accumulation of cadmium in the olfactory bulb. The cadmium concentration in the olfactory bulb was threefold greater than that in the remaining brain in rats injected SC with cadmium chloride ($CdCl_2$), at 0.5 mg/kg b.wt., for 25 weeks (40). Higher levels of cadmium were found in the olfactory bulb than in other brain region in adult male rats administered 100 ppm cadmium in the diet for 67 days (10).

These two lines of evidence suggest that chronic cadmium exposure might impair olfactory function. However, the role chronic exposure to cadmium plays in producing olfactory dysfunction has not been investigated experimentally. The major objectives of this study were: (1) to determine whether

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exposure of rats to chronic low levels of cadmium via inhalation alters the absolute olfactory threshold in a dose-related fashion; (2) to determine the extent of histological alterations of any cadmium-induced lesions in the nasal cavity, olfactory bulbs, and other target organs by using light microscopy; and (3) to correlate alterations in odor thresholds and nasal histopathology with cadmium content in the olfactory bulb. Two studies, each involving 20 weeks of exposure to CdO, were undertaken to address these issues.

METHOD

Overall Design

In the first of two separate experiments, the effects of CdO exposure on olfactory function were assessed by measurement of the absolute detection threshold, using a conditioned suppression paradigm. Other variables studied included renal function, level of cadmium in various tissues, pathology of lung, heart, kidney, and olfactory bulb, and the effect of cadmium on shock sensitivity. In the second study, olfactory function was again assessed by measurement of the absolute detection threshold. Damage to the nasal cavity resulting from exposure to cadmium was evaluated histologically. Finally, any latent effects of cadmium exposure on olfactory function were investigated during a 6-month recovery period.

Subjects

Adult male Long–Evans hooded rats, purchased from either the Charles River Laboratories (Experiment 1) or Harlan Industries (Experiment 2), were used. Rats were housed in suspended stainless steel cages and maintained in temperature (22 ± 2°C)-, humidity (50 ± 10%)-, and light (12 L : 12 D cycle)-controlled rooms. These facilities were fully AAALAC accredited and met or exceeded all NIH and USDA care guidelines. Access to water was restricted to maintain approximately 85% normal body weight to facilitate behavioral testing. Rats were fed Rodent Laboratory Chow 5001 (Ralston Purina Company, St. Louis, MO) ad lib. The animals were acclimated for 14 days prior to being introduced into the experiment.

Exposure

In each experiment, rats were randomly divided into three groups and exposed to target concentrations of 0, 250, or 500 $\mu g/m^3$ CdO via inhalation, 5 h/day, 5 days/week, for 20 weeks. The dose of 500 $\mu g/m^3$ was chosen as a ceiling dose because it was the highest level reported to produce slight renal damage [as reflected by cadmium content in kidney cortex of 200 $\mu g/g$ wet tissue, or proteinuria at a level of < 1 g protein per g creatinine (16)]. It was also 10 times the threshold limit value established for CdO.

Inhalation Exposure Techniques

CdO exposure was conducted in three 0.33-m^3 stainless steel and glass inhalation chambers. CdO was generated by nebulizing a solution of cadmium acetate [$Cd(CH_3COO)_2$] (Fisher Scientific Company, Fair Lawn, NJ), followed by thermally decomposing and oxidizing the resulting aerosol (27). The heated CdO-containing air stream then passed through a cooling coil, a water-cooled condenser, and a large flask for elimination of conglomerates. The electrical charge on generated particles was reduced by passing the air stream over a 2-μCi ^{85}Krypton radioactive source. The desired concentrations were achieved by adjusting the CdO-containing air stream as it entered the background air flowing into the exposure chamber. The flow of the background air stream—filtered for chemical, biological, and radiological contaminants—provided approximately 15 air changes per hour.

Cadmium aerosol concentrations in the exposure chambers were measured by collecting CdO on a 47-mm HA filter over a fixed period of time. A 4-h sample was obtained during each 5-h exposure run. Filters were wet ashed with nitric and hydrochloric acid and cadmium measured by a flame atomic absorption spectrophotometer (AAS) (Perkin-Elmer Model 403).

The size distribution of the aerosol in the chamber was characterized using two different techniques. The first employed an Andersen seven-stage cascade impactor with a 43-mm backup filter. A 4-h sample collected at the rate of 28.3 lpm was used for size determination. The amount of cadmium collected on each stage was weighed using a Mettler H542 analytical balance. The second approach involved placing Formvar®-coated grids inside the chambers during exposure and then determining particle frequency and size using electron microscopy.

Animals were positioned in the chamber to ensure equal exposure to CdO, remaining there from approximately 0900 to 1500 h without food or water. After inhalation exposure periods, animals were returned to housing units. Behavioral testing took place from 1600 to 2300 h. Animals were fed and watered at the conclusion of testing. Control animals received identical treatment as the experimental animals, but were exposed to filtered air only.

Olfactory Threshold Test

Conditioned suppression. Rats were trained on a conditioned suppression paradigm similar to that described by Smith (38) in preparation for odor threshold determination. Rats were water restricted for 23 h and then placed in Coulbourn E10-10 test cages. Each cage was enclosed by a sound-attenuated chamber. On one end of each test cage, a small breathing chamber (4 × 3 cm, 2.5 cm deep) was located 6 cm above the floor. Olfactory cues entered the breathing chamber through a port in the lower back wall and were exhausted through one at the top. A sipper tube was present, oriented in a way so that while licking, the rat received maximum exposure to any odor entering the chamber. Each rat was trained to lick the tube for water reinforcement in the presence of a flow of 2 l/min of clean air. After this response was learned by the rats, the schedule of reinforcement for the conditioned suppression paradigm was implemented. Although basically the same, there were slight variations in the protocols used in the two experiments. The primary differences were the use of a shorter duration for the variable interval (VI) reinforcement schedule (VI-2 min vs. VI-1 min) and a different odor stimulus [isoamyl acetate (IAA) vs. ethyl acetate (EA)] in Experiment 2.

Experiment 1. The reinforcement schedule for water was changed from continuous reinforcement used during shaping to a VI-10 s schedule, and gradually increased to a VI-2 min schedule. When the licking response was stable during daily 40-min sessions, the olfactory stimulus (3 ppm IAA) was presented for 20 s every 2–6 min (VI-4 min) until the rats habituated to the novelty of the stimulus. Each session thus consisted of 10 trials with an average intertrial interval of 4 min. Next, foot shock (0.26 mA for 0.5 s, Scientific Prototype 400 8J shock source) was generated at the offset of each odor stimu-

lus presentation until reliable suppression was obtained in response to the odor. The degree of suppression on a trial was measured by the Kamin (28) suppression ratio (SR), SR = $B/(A + B)$, where A is the number of licks observed in a 20-s period preceding the odor stimulus period, and B is the number of licks observed during the odor stimulus period. Using this formula, complete suppression yields a ratio of 0, whereas no suppression results in a value of 0.5. A suppression ratio ≤ 0.2 was considered the criterion for odor detection, because a well-trained rat usually continued licking a few times before suppressing the licking response upon detection of the odor.

When the criterion for suppression to the shock-reinforced IAA was reached (suppression ratio ≤ 0.2), threshold measurements to IAA were determined following the method described by Pierson (32) with some modifications. Following two initial trials of the training stimulus (3 ppm IAA), a series of four concentrations was presented in random order twice per daily session for 5 days. The stimulus interval among concentrations tested was 0.10 ppm; the lowest series of concentrations ranged from 0.10 to 0.50 ppm IAA. This procedure yielded 10 presentations per concentration, which were then averaged. In the next series of eight trials, a new set of four concentrations was used, preceded by two initial trials of lowest concentrations from the previous day's series. Series of concentrations were presented until a concentration that yielded a mean suppression ratio of 0.35 or more was obtained. The suppression ratios were then plotted against log odor concentration, a regression line calculated, and threshold determined. In this study, the threshold was considered the concentration at which the suppression ratio was 0.35, 0.35 being the midpoint of the suppression ratio for detection of the stimulus (0.2) and the ratio for no detection (0.5). During the exposure period, odor thresholds were determined after 10 and 20 weeks of exposure. Rats' performance of conditioned suppression to IAA was maintained by weekly training sessions using a suprathreshold stimulus.

Experiment 2. In the second experiment, the schedule of reinforcement for water was VI-1 min, and ethyl acetate served as the odorant. A final set of five odor concentrations (calculated to be 0.0016, 0.0048, 0.0145, 0.0447, and 0.3405 ppm) was presented in order of decreasing concentration along with three randomly inserted blank air stimuli during a 60-min testing session (VI-5 min schedule). Each odor concentration was presented twice during the session with the exception of the highest concentration, which was presented only once. Performance at each odor concentration was assessed by calculating a suppression ratio (26) based on the number of licks during a 20-s period immediately preceding the 20-s odor presentation (A) and the number of licks at the sipper tube during odor presentation (B), using the formula: SR = $A - B/A$ (0, no suppression; 1, complete suppression). Suppression ratios were plotted against log odor concentration, a regression line calculated, and threshold determined as the concentration at which the suppression ratio equalled 0.50. To more thoroughly investigate whether CdO exposure was affecting olfactory function at any time point, thresholds were obtained weekly. During the remaining daily sessions, the rats were trained on the conditioned suppression task using a single suprathreshold stimulus.

Buried Food Test

The possibility existed that a latency period was required before the toxic effects of cadmium were manifested (17,33). To test this hypothesis, 14 rats (seven control, three at 250 $\mu g/m^3$, and four at 500 $\mu g/m^3$) were trained on the buried food test (2,22) at the termination of exposure in Experiment 2, and their performance on this task tracked for the next 6 months.

Olfactometer

The basic design of the two olfactometers used to generate the olfactory stimuli in the two studies was essentially the same. Both were positive pressure, flow dilution olfactometers. All components of the olfactometers were constructed primarily of glass or Teflon. Filtered air provided by a compressed air supply system was used as the background air as well as the carrier stream for both odorant and blank stimuli. In the first stage of the olfactometers, the air stream was desiccated in a column containing anhydrous calcium sulfate and then filtered through a column of activated charcoal.

In the olfactometer used in Experiment 1, after filtration, the air stream was divided into three streams: carrier, dilution, and odorant streams. Air in the carrier stream (2 l/min) was led into the test chamber. The odor stimuli were generated by passing air (20 ml/min) over IAA contained in a diffusion tube maintained at a constant temperature (30°C). The odorized air was then diluted by the dilution air stream and was further divided into three channels, each carrying either 20%, 40%, or 40% of the air flow. Air from each channel was then led to the common port of a three-way Teflon solenoid valve. The normally open port of each valve was connected to a negative pressure exhaust port, and the normally closed port to the test chamber. Various concentrations of IAA were produced by energizing solenoid valves connected to one channel (20% or 40% flow), two channels (60% or 80% flow), or three channels (100% flow) to allow different concentrations of stimuli into the test chamber. Air flow in the carrier, dilution, and odorant stream were controlled by needle valves and monitored by flowmeters. The flowmeters were calibrated independently with a wet-test gas meter and a soap bubble flowmeter. The IAA concentrations were constantly monitored and verified by a Baseline Model 1030A gas chromatograph (GC) equipped with a flame ionization detector (Baseline Industries, Inc.).

In the second olfactometer, the odor stimuli were generated by passing air over ethyl acetate permeation tubes (VICI Metronics, Santa Clara, CA). Three permeation tubes, housed in glass holders maintained at a constant temperature (30°C), were used to generate stimuli over a 3 log-unit range. The odor stream from each permeation tube was divided into two channels by Teflon flow meters; one channel carried 33% of the flow (10 cc) and the other 67% (20 cc). This method allowed for the generation of nine ethyl acetate concentrations at $\approx$ ⅓ log steps (33%, 67%, and 100% of flow at each of three permeation tubes). A more complete description of this olfactometer can be found in Evans et al. (14).

Shock Threshold Test

Good performance on a conditioned suppression task based upon olfactory cues requires not only a functional olfactory system, but also intact cognitive processes and a stable foot shock threshold (3). Changes produced by cadmium exposure in either shock sensitivity or in cognitive functioning could potentially result in changes of conditioned suppression performance independent of any olfactory impairment. To insure any observed decrements in performance were not due to changes in shock sensitivity, flinch-jump tests were carried out periodically in Experiment 1. To make sure higher cogni-

tive processes were not impaired by CdO exposure, performance was monitored periodically on the conditioned suppression task using a suprathreshold stimulus in both Experiment 1 and 2.

The flinch-jump test was performed following the procedure described by Bonnet and Peterson (8). Rats were tested in a cage supported with a stainless steel rod grid floor and a house light, which was the only light source in the testing room. Shock was delivered by a Scientific Prototype 400 8J shock source for a 500-ms duration with a 20-s intershock interval. In the beginning of a test, the rat was allowed to adapt to the cage for 3–5 min. A single starting shock of 0.4 mA was delivered to reduce subsequent spontaneous motor activity and to provide a more uniform response set across animals for subsequent low-level shock presentation. Each rat was given three series of unavoidable shocks consisting of eight shocks of graded intensity. A flinch response consisted of elevation of one or two paws (usually front paws) whereas a jump response consisted of elevation of three or four paws. Flinch and jump thresholds were calculated for each animal as the lowest shock intensity at which the subject exhibited a flinch or jump response on at least three of the five series.

Renal Assessment

A set of 18 rats was randomly divided into three groups and exposed to 0, 250, or 500 $\mu g/m^3$ CdO for assessing renal function in Experiment 1. Rats were examined at the end of every 5 weeks (over the weekend) for the presence of proteinuria. Urine was collected overnight in stainless steel metabolism cages. To ensure obtaining sufficient urine samples, water-restricted rats were provided with water ad lib overnight before being transferred to metabolism cages. The concentration of total protein in the urine was determined by using N-Multistix reagent strips (Ames Division, Miles Laboratories, Inc., Elkhart, IN). Although the presence of proteinuria could be confounded by such factors as aging and activity levels, comparing the proteinuria levels of cadmium-exposed rats to those of control rats minimized differences due to these factors.

Cadmium Analyses in Tissues

A minimum of four rats per treatment group in Experiment 1 were euthanized every 5 weeks by IP injection of sodium pentobarbital (100 mg/kg) and perfused transcardially with saline solution. The olfactory epithelium, bulb, the remaining brain, lung, and kidney were removed and weighed. Pooling of tissue samples was required for AAS analysis of cadmium of both olfactory bulbs and brains.

Tissues were ashed in a muffle furnace at 500°C, digested with nitric acid, and the residues reconstituted in 10% nitric acid. The sample solutions and standards for cadmium were aspirated into a flame AAS (Perkin-Elmer Model 5000) equipped with a single-slot Boling burner. The analyses were performed at the 228.8-nm resonance line under conditions specified in the supplier's manual for analytical methods. For brain, olfactory epithelium, and bulb, aliquots of sample solutions were saved and extracted with methyl isobutyl ketone (47), and aspirated into the AAS.

Histopathology

In Experiment 1, the olfactory bulb, heart, lungs, and kidney were examined for cadmium-related histopathology. In Experiment 2, respiratory and olfactory epithelium of the nasal cavity were studied in detail.

Heart, lung, and kidney. Six rats (two control, two at 250 $\mu g/m^3$, and two at 500 $\mu g/m^3$) at 15 weeks of exposure and 17 rats (five control, seven at 250 $\mu g/m^3$, and five at 500 $\mu g/m^3$) at 20 weeks of exposure in Experiment 1 were subjected to routine histological examination of the heart, lung, and kidney. The rats were euthanized with an IP injection of sodium pentobarbital (100 mg/kg) and tissues fixed by immersion in 10% neutral-buffered formalin, Next, the tissues were embedded in paraffin, cut at 6 μm, dehydrated in an ascending series of ethanols and xylene, and stained with hematoxylin and eosin.

Olfactory bulb. The olfactory bulbs of four rats from each treatment group in Experiment 1 were examined using Nissl staining (cytoarchitectural differences) (44).

Rats were euthanized, perfused transcardially with saline, followed by fixation with 10% paraformaldehyde and 1.25% glutaraldehyde, and a 10% sucrose solution. The bulbs were removed, weighed, and placed in 20% sucrose storage solution overnight, then embedded in albumin gelatin and sectioned coronally on a freezing microtome at a thickness of 40 μm. The sections were mounted onto glass slides, stained with cresyl violet for Nissl substance, and dehydrated in an ascending series of ethanols and xylene. The sections were rinsed with distilled water, mounted onto glass slides, and dehydrated in an ascending series of alcohol and xylene.

Nasal cavity. Nasal pathology resulting from CdO exposure was studied in Experiment 2. Rats were euthanized at 1, 5, and 10 days after commencement of exposure; after 10 and 20 weeks of exposure; and 3 and 6 months postexposure. A phosphate-buffered 4% paraformaldehyde fluid was instilled into the nares of the rats after euthanasia, but before dissection. The nasal septum and turbinates were place in phosphate-buffered 2.0% paraformaldehyde/2.5% glutaraldehyde, an isoosmolar fluid with calcium salts and dextrose, for at least 24 h (22). After postfixation in 1% osmium tetroxide for 2 h, dehydration in an ascending series of ethanols and propylene oxide, the tissue was embedded in Spurr. Sections 1 μm thick were placed on glass slides and stained with toluidine blue. Overall mucosal thickness, thickness of the lamina propria, respiratory and olfactory epithelia were measured by a line drawn perpendicular to the basement membrane, using a Zeiss Photomik III with camera lucida and SIGMA SCAN imaging software (Jandel Scientific). The number of sensory bipolar cells, globose cells, and sustentacular cells, per 100 μm of basement membrane, was determined. Other cell types in the sensory epithelium, such as dark basal cells, microvillar cells, and inflammatory cells, were assessed separately. The percentage of each these cell types occupied in the whole epithelium in relation to the total thickness of the epithelium was determined. The relative thickness of the epithelium and the subadjacent lamina propria was also compared.

Neutrophilic and eosinophilic leukocytes, mast cells, and small and large mononuclear cells counted per 1250 × field in the lamina propria beneath the epithelium were used as an index of inflammation/damage.

RESULTS

Aerometry

The mean cadmium concentrations in the chambers during the 20 weeks of exposure in Experiment 1 were 662.7 ± 24.2 and 325.6 ± 21.4 $\mu g/m^3$ as analyzed by AAS. Although the actual mean cadmium concentrations were higher than the target concentrations, cadmium analysis of the kidney at week 5 revealed low levels of cadmium accumulation. Conse-

quently, no attempt was made to reduce the exposure levels. The mean cadmium concentrations in the chambers during Experiment 2 were 550 ± 24.2 and 200.5 ± 7.4 $\mu g/m^3$. The mass median aerodynamic diameter of the CdO aerosol generated by the exposure system was found to be 0.50 μm with a geometric standard deviation of 1.80 using the Andersen impactor and 0.74 μm ± 2.6 using electron microscopy. These values are in close agreement with other studies using the same method for generating CdO aerosols (30).

Gross Observations

Exposure of adult rats to nominal 250 or 500 $\mu g/m^3$ CdO aerosol for 20 weeks did not result in mortality or any overt signs of morbidity in either study. Body weight gain remained constant during the 20 weeks of exposure to CdO. It should be pointed out that weight was controlled (by water restriction) for those rats undergoing olfactory testing. However, no differences in the body weight gain were observed in a subgroup of subjects that were not water restricted and were also exposed to 250 or 500 $\mu g/m^3$ CdO.

Cadmium Analyses in Tissues

The levels of cadmium in various tissues are presented in Figs. 1 and 2 and Tables 1-3. As shown in Fig. 1, the increase of the cadmium in the kidney was both dose and time dependent. ANOVA revealed significant treatment, $F(2, 36) = 192.59$, $p < 0.01$, and time effects, $F(3, 36) = 85.32$, $p < 0.01$. For the subgroup of rats supplied with water ad lib, kidney cadmium levels were found to be no different than the levels in the water-deprived group, suggesting that water deprivation did not affect renal uptake of cadmium.

Only the lungs of the rats exposed for 15 and 20 weeks were analyzed for cadmium levels. The accumulation of cadmium in the lung (Table 1) was dose dependent, $F(2, 17) = 114.2$, $p < 0.01$; however, the level of cadmium in the lungs of rats exposed to either dose level did not continue to increase from 15 to 20 weeks of exposure (i.e., the accumulated lung burden was not directly proportional to exposure concentration).

Within the olfactory system, only the olfactory mucosa of the rats exposed for 20 weeks was analyzed. There was a substantial amount of cadmium in the olfactory mucosa of rats exposed to CdO (Table 2), with uptake significantly elevated with increasing dose, $F(2, 12) = 179.2$, $p < 0.01$. The cadmium levels in the olfactory epithelium equaled those in the kidney. The olfactory bulbs from exposed animals accumulated a substantial amount of cadmium that was both dose dependent, $F(2, 11) = 34.26$, $p < 0.01$, and time dependent, $F(3, 11) = 22.61$, $p < 0.01$ (Fig. 2). As shown in Table 3, the brain contained the lowest level of cadmium all of tissues analyzed. (Given the small sample size, cadmium levels in the brain should only be viewed as approximate values.) At both low and high doses of cadmium, levels in the brain increased slightly after 5 weeks of exposure where they remained through exposure week 10. Although at week 20 these levels increased twofold, this was minor compared to the change in other tissues.

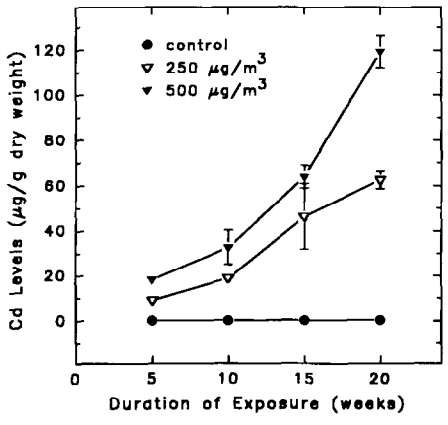

FIG. 1. Kidney cadmium levels (±SE) in rats exposed to 0, 250, or 500 $\mu g/m^3$ CdO for 5, 10, 15, or 20 weeks. Error bars not depicted are smaller than the symbol. $n = 4$ for 5- and 10-week values; $n = 2$ for 15-week values; $n = 5$-7 for 20-week values.

TABLE 1

CADMIUM LEVELS IN THE LUNG ($\mu g/g$ DRY WEIGHT) OF RATS EXPOSED TO CdO FOR 1 OR 20 WEEKS

	15 weeks ($n = 2$)	20 weeks ($n = 6$)
Control	0.58 ± 0.13	0.53 ± 0.19
250 $\mu g/m^3$ CdO	132.0	143.3 ± 10.3
500 $\mu g/m^3$ CdO	199.0 ± 8.0	181.0 ± 13.5

Values are mean ± SE.

Renal Assessment

ANOVA failed to reveal either treatment or time effects in kidney function; proteinuria was unaffected by either cadmium exposure for 20 weeks or aging. Rats exposed to either level of CdO developed a diffuse and reversible degeneration in the epithelium of the proximal convoluted tubules. The results from kidney histology were consistent with those from the kidney function test in that slight, reversible tubular degeneration is not expected to produce any functional deficits.

Flinch-Jump Test

Neither flinch nor jump thresholds were affected by cadmium exposure or aging. Repeat measure ANOVA did not reveal any treatment or time effect on either threshold, suggesting that the shock sensitivity of rats remained unaffected throughout the entire study.

Histopathology

Cytoarchitectural differences of the olfactory bulbs of control and cadmium-exposed rats were not apparent in Nissl-

TABLE 2

CADMIUM LEVELS IN THE OLFACTORY MUCOSA ($\mu g/g$ DRY WEIGHT) OF RATS EXPOSED TO CdO FOR 20 WEEKS

Control ($n = 5$)	0.52 ± 0.09
250 $\mu g/m^3$ CdO ($n = 6$)	75.8 ± 3.0
500 $\mu g/m^3$ CdO ($n = 4$)	135.0 ± 9.6

Values are mean ± SE.

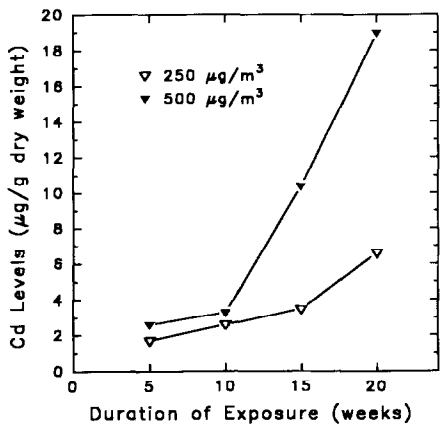

FIG. 2. Cadmium levels in the olfactory bulbs of rats exposed to 0 (below detection limits), 250 or 500 $\mu g/m^3$ CdO for 5, 10, 15, or 20 weeks. Two olfactory bulbs were pooled/sample for AAS analysis. n = 2 for 5- and 10-week values, n = 1 for 15-week value; n = 3 for 20-week value.

stained sections, nor did the cross-sectional area of the bulb of the controls differ from those of the exposed rats.

Neither the olfactory nor respiratory epithelia varied in thickness after exposure to 250 or 500 $\mu g/m^3$ of CdO for 20 weeks. After cadmium treatment, however, there was a slight increase in thickness of apical cytoplasm of the sustentacular cells, which began at about 10 days after treatment and continued on throughout the exposure period. Olfactory epithelium also tended to be increased in thickness, in the immediate postexposure period, and in the animals that recovered for extended periods of time, but the change was not significant.

Counts of the percentage of bipolar cells in the olfactory epithelium were also not statistically significant from controls counts, nor was there an alteration in the percentage of sustentacular cells in the epithelium. Globose basal cells were not increased and the number of microvillar cells in the superficial olfactory epithelium was not different from controls. The ratio of olfactory knobs to bipolar cells, although elevated at two time points after exposure to 500 $\mu g/m^3$, was not statistically significant. To investigate whether the initial exposure to CdO caused tissue damage in the nasal cavity, which subsequently recovered with time [as reported in the lungs (20)], the olfactory mucosa was examined after only 1, 5, or 10 days of exposure. No treatment-related changes were found. There was essentially no inflammation in the lamina propria or olfactory epithelium after exposure to 250 or 500 $\mu g/m^3$ CdO, whether just after exposure or during recovery.

The lungs of the rat exposed to 250 $\mu g/m^3$ CdO showed a slight fibrosis in the wall of the terminal bronchioles. The bronchiolar epithelium was slightly hyperplastic. There was an accumulation of macrophages in the alveolar spaces. These macrophages had a foamy cytoplasm composed of proteinaceous and lipid materials. The proteinaceous material was finely granular whereas the lipid material appeared as well-demarcated vacuoles. The rats exposed to 500 $\mu g/m^3$ CdO developed more severe pulmonary lesions, including thickening of the alveolar wall, accumulation of macrophages in alveolar spaces, and hyperplasia in the epithelium of the terminal bronchioles with papillary formation. Emphysema was present in some rats, as demonstrated by the enlarged alveolar space (Table 4).

The hearts from rats exposed to either 250 and 500 $\mu g/m^3$ CdO showed a degenerative cardiomyopathy of moderate to marked degree. Muscle fibrils and filaments appeared as granular material, or as vacuolar structures, and later as scar tissue (Table 4).

Olfactory Detection Threshold

Exposure of rats to 250 or 500 $\mu g/m^3$ CdO aerosol for 20 weeks did not result in anosmia in either experiment. This was determined by monitoring performance on the conditioned suppression task using a suprathreshold odorant stimulus; all rats were able to detect the suprathreshold stimuli throughout the entire experiment. The unperturbed performance on the conditioned suppression task using a suprathreshold stimulus also suggested that cognitive function in the rat was unaffected by cadmium exposure.

In both Experiments 1 and 2, ANOVA with repeated measures revealed no treatment-related changes in the olfactory thresholds. A summary of threshold data is presented in Fig. 3 (Experiment 1) and Fig. 4 (Experiment 2). The thresholds did fluctuate slightly over time, however. In Experiment 1, for all three treatment groups, the detection thresholds increased at 10 weeks and then decreased to near the baseline level at the end of week 20. In Experiment 2, the threshold values, obtained weekly, were more stable than in the first experiment. (Due to equipment malfunction, the data collected during weeks 7 and 8 were spurious for all groups and were omitted from analysis.) Again, no treatment-related differences in threshold were observed.

Buried Food Task

All rats were able to consistently find the buried food within 1 min or less, suggesting that at least a rudimentary sense of olfaction was intact throughout the 6 months after termination of exposure. Thus, there was no evidence of increased cadmium toxicity after a latent period.

DISCUSSION

Cadmium has been implicated as being toxic to the olfactory system. It has been reported that workers in alkaline battery plants, zinc refineries, and cadmium smelters developed anosmia after prolonged exposure to cadmium (1,6,29, 33,36,41,42). These workers had been exposed to various forms and amounts of airborne cadmium, ranging from 0.07 to 15 mg/m^3. Chronic administration of cadmium to adult rats orally or subcutaneously has been found to cause a significant increase in the accumulation of cadmium by the olfactory bulb (10,40). These two lines of evidence suggested that chronic cadmium exposure might impair the olfactory sense. The present study, using inhalation exposure of CdO, an ex-

TABLE 3

CADMIUM LEVELS IN THE BRAIN ($\mu g/g$ DRY WEIGHT) OF RATS EXPOSED TO CdO FOR 5, 10, OR 20 WEEKS

	5 weeks*	10 weeks*	20 weeks†
Control	0.04	0.05	0.08
250 $\mu g/m^3$ CdO	0.07	0.07	0.13
500 $\mu g/m^3$ CdO	0.08	0.09	0.18

*N = 1, four samples were pooled for AAS analysis.
†N = 2, three samples were pooled for AAS analysis.

TABLE 4

HISTOLOGIC CHANGES IN THE RATS EXPOSED TO FILTERED AIR OR CdO

	Lung			Heart		
ID	Lipoproteinosis	Hyperplasia	Fibrosis	Perivascular Edema	Myocarditis	Degeneration
			Filtered Air			
11						
37						
42						
49						
72						+
			250 $\mu g/m^3$ CdO			
54	+ +	+				+
08	+ +	+			+	+
25	+	+				
64	+	+				+ +
57	+ + +	+				+ +
47	+ +	+ +	+			+
65	squamous carcinoma			+ +		+
			500 $\mu g/m^3$ CdO			
55	+ +	+	+	+ +		+
32	+ + +	+ +	+ +	+		+ +
74	+ + +	+ +	+ +			+
65				+ +		+
68	+	+	+			

posure route relevant to the occupational situation, was the first attempt to experimentally demonstrate cadmium-induced olfactory dysfunction with a rat model.

In this study, rats were exposed to target levels of 250 or 500 $\mu g/m^3$ CdO aerosol for 20 weeks in two separate experiments. Cadmium levels in various tissues indicate that, in addition to the kidney and lung serving as target organs for cadmium, cadmium also accumulates in the olfactory epithelium and olfactory bulb. At the exposure levels employed, both lung and cardiac pathology were observed; slight renal pathology was not accompanied by proteinuria. Because an increase in proteinuria is a sensitive measure of renal dysfunction produced by cadmium (15–17), the present finding of no increase in proteinuria suggests no cadmium-induced kidney dysfunction. This finding is consistent with the study by Prigge (34), in that CdO inhalation exposure did not increase proteinuria in the rat, whereas oral exposure to $CdCl_2$ for 90 days did.

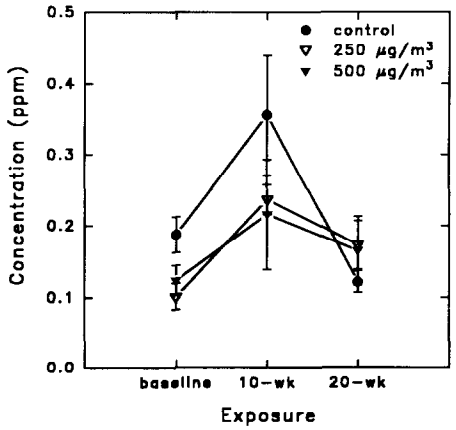

FIG. 3. Absolute thresholds (mean ± SE) for detection of isoamyl acetate by rats exposed to 0, 250, or 500 $\mu g/m^3$ CdO.

The recent publication of a study that investigated the disposition of inhaled CdO in the rat (11) reported results that were in close agreement with findings of the current study. First, accumulation of cadmium in the lung resulting from nearly equivalent CdO exposure conditions (250 $\mu g/m^3$ for 93 days vs. 250 $\mu g/m^3$ for 105 days) resulted in comparable tissue concentrations (after adjustment for differences between wet and dry weights). Secondly, both studies found that accumulated lung burden was not directly proportional to exposure concentration. Finally, cadmium concentration in the kidney was roughly equivalent in the two studies, when similar exposure groups were compared. Dill et al. (11) also concluded that cadmium exposure via inhalation at these doses should not produce kidney damage.

The cadmium level in the olfactory bulbs of rats exposed to 500 $\mu g/m^3$ for 20 weeks was 19.0 $\mu g/g$ dry weight, which is much higher than levels found in other studies (10,40). Because the present study employed inhalation exposure, whereas other investigations used oral or parenteral administration, it is likely that inhalation exposure is a far more effective route for cadmium to gain entry into the CNS, particularly the olfactory bulb. On the other hand, the cadmium level in the brain obtained in the present study was as low as that reported by other studies, suggesting that overall brain uptake of cadmium was not facilitated by inhalation exposure.

The particles of CdO used in the present study are considered respirable particles (< 1.0 μm) (35), as confirmed by the high concentration of cadmium found in the lungs of exposed

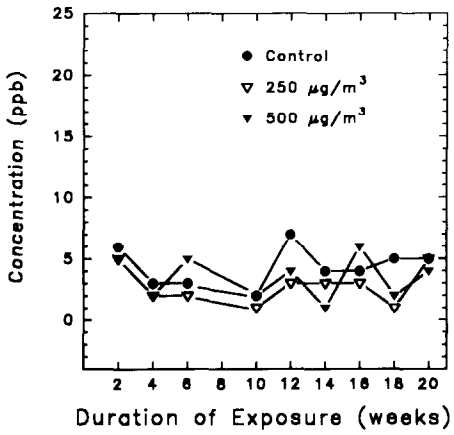

FIG. 4. Absolute thresholds for detection of ethyl acetate by rats exposed to 0, 250, or 500 $\mu g/m^3$. Each point represents the average of 2-week data.

animals. That these particles gained access to the olfactory mucosa is indicated by the increased concentration of cadmium found in this tissue. Recent studies have found that, contrary to conventional belief, a large percentage of ultrafine aerosols is also deposited in the nasal passages and not only in the deep recesses of the lungs (46). Such a finding is especially relevant in the case of metal fumes, which are often composed of extremely fine aerosols. Overall, these results suggest that not only are molecules from volatile compounds (<0.004 μm) deposited in the olfactory epithelium, but also respirable particles of the size generated in this study.

The accumulation of cadmium in the olfactory bulb in the present study is greater than that reported by other studies, and might be explained by an additional route of cadmium transport to the CNS—axonal transport from the olfactory epithelium to the olfactory bulb. It has been demonstrated that wheat germ agglutinin (WGA) or WGA conjugated to horseradish peroxidase (HRP) can be transported transneuronally in the CNS (5,37,39). In the olfactory pathway, Shipley (37) has demonstrated that WGA-HRP can be transported from the olfactory epithelium to the olfactory bulb and more centrally located olfactory structures. Thus, axonal transport is a pathway by which toxins may reach the CNS through the olfactory receptor neurons (21). It is by this mechanism that such heavy metals as iron-Dextran (31), lead (6), and thallium (7) are transported in axons. Hastings and Evans (23) instilled radiolabeled cadmium unilaterally in the rat naris and found cadmium in the olfactory bulb ipsilateral to the site of exposure, but not in the contralateral side. Furthermore, accumulation in the olfactory bulb was greatest when exposure was via the nasal route (12). Thus, by axonal transport, cadmium and other metals may bypass the blood–brain barrier and gain entry into the CNS. Because the olfactory mucosa is in direct contact with airborne particles, this neuronal transport mechanism may provide an additional portal of entry for toxic compounds to the olfactory bulb and other parts of the brain when the exposure route is via inhalation.

Because a significant amount of cadmium is deposited on the olfactory epithelium, it seems likely that olfaction would be compromised. However, the present findings indicate that exposure of rats to chronic low level of CdO aerosol does not result in anosmia, because there was no adverse effect on performance of the conditioned suppression task. Furthermore, olfactory absolute threshold levels, a more sensitive measure of olfactory function, were also unaffected after 20 weeks of cadmium exposure.

No histopathological lesions were found in the olfactory bulb when examined by light microscopy, nor was the size of the olfactory bulb altered by cadmium exposure. Long-term exposure to 500 $\mu g/m^3$ CdO did not result in any structural damage to the olfactory mucosa, either. The possibility cannot be ruled out, however, that there were some ultrastructural changes in the olfactory system that could not be detected by the techniques used for this assessment.

The lack of an adverse effect on the olfactory mucosa cannot be attributed to the possibility that the CdO particles failed to come into contact with the tissue due to particle size, flow patterns, etc., because one of the highest concentration of cadmium in the tissues analyzed was found in the olfactory mucosa. That the form of cadmium employed was low in toxicity is not supported either, because 1) CdO has been linked with olfactory dysfunction in several studies in the literature (36,42), and 2) inhaled CdO is rapidly converted to a more soluble form of Cd, at least in the lungs (19). Although exposure to a higher concentration of cadmium might have resulted in nasal toxicity and olfactory dysfunction, the levels employed were sufficient to produce both lung and cardiac toxicity within the same time period. Thus, exposure to higher concentrations of cadmium was not warranted.

Failure to produce any alterations in the olfactory function by CdO inhalation exposure may be due to the following reasons:

1. *Other metals:* Nickel, present in the cadmium battery plant, has been implicated as being toxic to the olfactory system. In Friberg's report (17), 37% of cadmium-exposed workers who developed anosmia were also exposed to nickel dust. The alkaline battery workers who developed anosmia after chronic exposure to CdO dusts, as reported by Adams and Crabtree (1), had also been exposed to nickel dust. Others (29,42), however, reported impaired olfaction in workers exposed to cadmium at a zinc refinery, where no concomitant nickel exposure was encountered. In a study designed specifically to investigate the effects of inhalation of nickel on olfactory function, no functional effects were evident, although structural damage in the olfactory mucosa was observed (14). It is still possible that there may be some synergy between cadmium and nickel (or some other metal) that results in enhanced toxicity to the olfactory system.
2. *Role of metallothionein (Mt):* Metallothionein has been proposed to play an important role in cadmium detoxification. It has been postulated that nearly all of the cadmium in cells is bound to Mt and it may therefore protect the cell contents from potential toxic effects of cadmium ion (43). Mt is a rather ubiquitous, intracellular protein present in various tissues of normal animals, including the brain (9). It has been demonstrated that Mt synthesis can be induced in the brain of rats injected SC with $CdCl_2$, although the concentrations are quite low when compared to those in other tissues. Whether sufficient Mt is present in the brain to protect against cadmium is unknown.
3. *Duration of cadmium exposure and age:* Clinical reports have shown that the percentage of workers who developed anosmia correlated well with the duration of cadmium exposure (29,33). Moreover, the incidence of anosmia in the exposed population was restricted almost exclusively to the

older or retired worker. Thus, the above studies suggest two points: 1) the longer the exposure, the more likely it is for one to develop smell dysfunction; and 2) the older the worker, the more likely smell dysfunction will occur, possibly because cadmium exposure is an additional stressor to the already aging olfactory system.

The workers reported in the above clinical studies who developed anosmia were exposed to cadmium for an average of 20 years. In the present study, rats were exposed to cadmium for 20 weeks. Olfactory function testing carried out during the exposure period was not affected. When testing was continued for 6 months postexposure in Experiment 2, there was still no evidence of diminished olfactory function. Although it does not appear that cadmium has a long-term cumulative effect in the rat olfactory system, an alternate explanation is that there is not a parallel decline in olfactory function that is observed in humans.

4. *The role of reconstitution:* The olfactory sensory neurons in the vertebrate central nervous system are unique in that they undergo continuous neurogenesis and replacement both in normal circumstances (turnover) and after experimental intervention (18). (Although such reconstitution occurs in the human olfactory system, it is much more robust in macrosmatic animals such as rodents.) Anatomical, physiological, and behavioral studies have shown that transection of the olfactory nerves leads to a degeneration of sensory neurons, followed by a neurogenesis and replacement with newly formed cells as well as by the reestablishment of functional connections of the nerve with the olfactory bulb (18). In rats, it has been demonstrated that the ability to perform in an odor detection task is restored in about 5–7 days after the destruction of the olfactory epithelium (2,22). In fact, the olfactory mucosa of rodents can suffer extensive damage and then support normal olfactory function long before morphological recovery (13,22). Thus, it is not surprising that dysfunction of the olfactory system did not occur after exposure to cadmium, because there was little or no adverse effect on the olfactory mucosa. Only when the Bowman's glands within the mucosa are damaged are persistent olfactory deficits observed (24,25).

In summary, failure to induce olfactory damage by cadmium exposure may be due to the role of Mt in cadmium detoxification, the duration of exposure and/or the effects of aging, reconstitution of the olfactory system, or some combination of the above. The finding of high levels of cadmium in the olfactory bulbs after inhalation exposure, however, warrants further examination of the relationship between the entry of cadmium into the CNS and human neurodegenerative diseases. Finally, given the resiliency of the rodent olfactory system to toxic insult, a more proper animal model for the study of olfactotoxins would be a microsmatic animal, such as nonhuman primates.

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