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## Two-Week, Repeated Inhalation Exposure of F344/N Rats and B6C3F<sub>1</sub> Mice to Ferrocene<sup>1</sup>

J. D. SUN,<sup>2</sup> A. R. DAHL, N. A. GILLETT, E. B. BARR, M. L. CREWS, A. F. EIDSON, W. E. BECHTOLD, D. G. BURT, M. P. DIETER,<sup>3</sup> AND C. H. HOBBS

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*Received August 14, 1990; accepted January 31, 1991*

Two-Week, Repeated Inhalation Exposure of F344/N Rats and B6C3F<sub>1</sub> Mice to Ferrocene. SUN, J. D., DAHL, A. R., GILLETT, N. A., BARR, E. B., CREWS, M. L., EIDSON, A. F., BECHTOLD, W. E., BURT, D. G., DIETER, M. P., AND HOBBS, C. H. (1991). *Fundam. Appl. Toxicol.* 17, 150-158. Ferrocene (dicyclopentadienyl iron; CAS No. 102-54-5) is a relatively volatile, organometallic compound used as a chemical intermediate, a catalyst, and as an antiknock additive in gasoline. It is of particular interest because of its structural similarities to other metallocenes that have been shown to be carcinogenic. F344/N rats and B6C3F<sub>1</sub> mice were exposed to 0, 2.5, 5.0, 10, 20, and 40 mg ferrocene vapor/m<sup>3</sup>, 6 hr/day for 2 weeks. During these exposures, there were no mortality and no observable clinical signs of ferrocene-related toxicity in any of the animals. At the end of the exposures, male rats exposed to the highest level of ferrocene had decreased body-weight gains relative to the weight gained by filtered air-exposed control rats, while body-weight gains for all groups of both ferrocene- and filtered air-exposed female rats were similar. Male mice exposed to the highest level of ferrocene also had decreased body-weight gains, relative to controls, while female mice had relative decreases in body-weight gains at the three highest exposure levels. Male rats had a slight decrease in relative liver weight at the highest level of exposure, whereas no relative differences in organ weights were seen in female rats. Male mice had exposure-relative decreases in liver and spleen weights, and an increase in thymus weights, relative to controls. For female mice, relative decreases in organ weights were seen for brain, liver, and spleen. No exposure-related gross lesions were seen in any of the rats or mice at necropsy. Histopathological examination was done only on the nasal turbinates, lungs, liver, and spleen. The only exposure-related finding was histopathologic lesions in the nasal turbinates of both species. These lesions were primarily centered in the olfactory epithelium and were morphologically diagnosed as subacute, necrotizing inflammation. Nasal lesions were observed in all ferrocene-exposed animals and differed only in severity, which was dependent on the exposure concentration. *In vitro* metabolism studies of ferrocene showed that nasal tissue, particularly the olfactory epithelium, had ~10 times higher "ferrocene hydroxylating" activity than did liver tissue from the same animals. These results suggest that the mechanism of ferrocene toxicity may be the intracellular release of ferrous ion through ferrocene metabolism, followed by iron-catalyzed lipid peroxidation of cellular membranes.

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Ferrocene (dicyclopentadienyl iron; CAS No. 102-54-5) is a relatively volatile, organometallic compound consisting of a ferrous ion bound between two cyclopentadienyl rings. It is increasingly used as a chemical intermediate, a catalyst, and as an antiknock additive in gasoline. Ferrocene, like a number of other organometallic compounds, is also used increasingly in industry as an ultraviolet stabi-

lizer, a smoke suppressant for polymers, and a polymerization catalyst, as well as in lubricant additives, and rocket propellants. The annual production of ferrocene is reported to be less than 1000 pounds per year (USEPA, 1982).

Ferrocene was selected for study by the National Toxicology Program because of its structural similarities to other metallocenes, some of which are carcinogenic, and because of the increasing potential for human exposure to ferrocene. The current TLV for ferrocene is  $10 \text{ mg/m}^3$  (ACGIH, 1986). At temperatures of  $20^\circ\text{C}$  or higher and concentrations in air of less than  $40 \text{ mg/m}^3$ , ferrocene exists mostly as a vapor (Jacobs *et al.*, 1983). Therefore, ferrocene vapor, as opposed to droplets or particles, is the most likely chemical form to be inhaled by people.

Despite the increasing use of ferrocene and similar organometallic compounds, there is virtually no information available regarding the effects of long-term inhalation of vapors or aerosols of such compounds. Inhalation exposures to inorganic forms of iron have been shown to be relatively nontoxic (Committee on Medical and Biological Effects of Environmental Pollutants, Subcommittee on Iron, 1979). However, because of the lipophilicity imparted by its organometallic structure, ferrocene is able to deliver iron to lipophilic, intracellular sites in tissues which neither insoluble or water-soluble iron can reach (Dahl and Briner, 1980). As a result, there may be iron-induced toxic effects that are not elicited by inorganic forms of iron, because of the differences in microdosimetry at specific sites.

Dahl and Briner (1980) studied the toxicokinetics of inhaled ferrocene vapor. For these studies, they used ferrocene that was labeled with both iron-59 and tritium. Inhalation exposure to ferrocene vapor resulted in an initial deposition of 37% of the inhaled vapor. Of this amount, approximately 60% was deposited in the nasopharyngeal region and 25% in the lung. Over 75% of the tritium label was excreted within the first day, but the  $^{59}\text{Fe}$  label remained, largely in the bronchopul-

monary and nasopharyngeal regions, over the 117-day duration of the study. This suggested that the ferrocene was being metabolized in the respiratory tract, probably to hydroxyferrocene, which then decomposed in the lipid-containing environment of the cellular endoplasmic reticulum. These investigators hypothesized that, because of the tendency of iron ions to catalyze the production of hydroxy radicals, inhaling ferrocene created the potential for cellular injury in the respiratory tract, particularly at the sites where ferrocene could deliver its iron moiety. By using an estimated half-time of 200 days for clearance of iron introduced into rat lungs by inhaled ferrocene, Dahl and Briner calculated that a human breathing ferrocene at the TLV concentration of  $10 \text{ mg/m}^3$  would have acquired 1.7 g of ferrocene-derived iron in the pulmonary region at equilibrium.

The purpose of our study was to characterize the toxic effects of inhaled ferrocene vapor after 2-weeks of inhalation exposure. We also investigated the *in vitro* metabolism of ferrocene in selected tissues to gain further insight into the importance of metabolism of this compound as a mechanism of ferrocene-induced toxicity.

## MATERIALS AND METHODS

*Chemicals.* The ferrocene used for this study was obtained from Midwest Research Institute and was >99% pure, as determined by high-performance liquid chromatography (HPLC). We studied the potential for ferrocene degradation that might be caused by the generation of exposure atmospheres. HPLC analyses and graphite furnace atomic absorption of the exposure atmosphere showed that ferrocene was degraded by less than 1%.

$^{59}\text{Fe}$ -labeled ferrocene was prepared by treating a mixture of anhydrous ferrous chloride and  $^{59}\text{Fe}$ -labeled ferric chloride with sodium cyclopentadienide. The labeled ferrocene was purified by sublimation and then was dissolved in ether. The concentration of ferrocene in the ethereal solution was determined by measuring the visible absorbance at 440 nm and calculating the concentration, using the molar absorptivity ( $0.95 \text{ m}^{-1}\text{cm}^{-1}$ ). The  $^{59}\text{Fe}$  activity was determined by scintillation counting. From the concentration and activity of the  $^{59}\text{Fe}$ , we were able to determine the specific activity of the synthesized material, which was

then corrected for loss of activity due to radiodecay; the half-life of  $^{59}\text{Fe}$  is 45 days.

*Exposure atmosphere generation and exposure system.* Wright dust feeders (WDF) (BGI, Inc., Waltham, MA) were used to generate the ferrocene aerosols. The aerosol generated by each WDF was heated to 200°C to vaporize the ferrocene particles. A separate WDF generator/vapor system was used for each of the five exposure chambers. Six Hazleton H-2000 whole-body exposure chambers were used to house animals being exposed to each of the five levels of ferrocene (2.5, 5.0, 10, 20, and 40 mg ferrocene vapor/m<sup>3</sup>) or to filtered air (control). The highest ferrocene exposure level represented the highest concentration of ferrocene vapor that could be generated without producing condensation aerosols of the vapor in the chamber.

The method used to determine aerosol concentrations in the chambers was a bubbler sampling train. The hexane solution from each bubbler system was analyzed on a Hewlett-Packard Model HP5890A gas chromatograph to determine the chamber vapor concentration. A real-time aerosol monitor (RAM-S; GCA Corp., Bedford, MA) was used to determine whether significant levels of particles were present in the chambers during exposures. During the exposures, we measured the homogeneity of the ferrocene vapor within each exposure chamber by injecting heated line samples directly into the gas chromatograph.

*Animals.* Thirty male and 30 female F344/N rats and 30 male and 30 female B6C3F<sub>1</sub> mice (4 weeks old) were received from the Simonsen Laboratories, Inc. (Gilroy, CA). The animals were quarantined in cage units within a Hazleton H-2000 chamber prior to exposure, 2 per cage, for the first 4 days and then 1 per cage for 11 (rats) or 12 (mice) days. Animals were exposed for 5 days/week, starting on a Monday, for 2 weeks, with 2 or 3 additional exposure days before terminal euthanization, for a total of 12 or 13 exposure days for both rats and mice. This exposure schedule was necessitated to accomplish day-before-euthanization exposure and to facilitate timely necropsy of euthanized animals. Most animals (67%) were exposed for 12 days.

Prior to the start of exposures, we used a computer-based randomization system (Path/Tox, Xybio Corp.) to randomly assign the rats and mice. Individual animals assigned to the study were identified by tail tattoos for rats and tail tattoos and ear tags for mice. Serum was obtained from two males and two females of each rodent species prior to the first day of exposure and was analyzed for antibody titers to specific rat viruses (Kilham rat virus, Toolan H-1 virus, pneumonia virus of mice, Sendai virus, rat coronavirus/sialodacryoadenitis virus) and mouse viruses (K. virus, polyoma virus, mouse hepatitis virus, ectromelia, mouse encephalomyelitis (Theiler's GD VII), mouse adenovirus, lymphocytic choriomeningitis virus, Minute virus of mice, Sendai virus, pneumonia virus of mice, Epizootic diarrhea of infant mice, and Reovirus 3). Titers were negative for all of these agents.

The animals were fed Zeigler NIH-07 Open Formula Rat or Mouse Ration (Zeigler Brothers, Inc., Gardners,

PA). Food was withheld during the 6-hr-exposure period. Water was available at all times, provided by an automatic watering system. Temperature and relative humidity in the inhalation chambers were monitored once every 5 min and averaged  $22.8 \pm 1.1^\circ\text{C}$  and  $55 \pm 6\%$  (mean  $\pm$  SD), respectively. The light cycle was automatically controlled to provide 12 hr of fluorescent light and 12 hr of darkness each 24 hr (on at 6 AM and off at 6 PM).

*Experimental design.* Rats and mice were weighed prior to starting the study, after 1 week of exposure, and at terminal euthanization. Detailed clinical examinations were also made at these times. Morbidity/mortality checks were made twice daily on all animals. Rats and mice were killed at terminal euthanization by cardiac puncture exsanguination while under halothane anesthesia, and each animal was given a complete gross necropsy examination. The liver, thymus, right kidney, right testicle, brain, heart, and lungs were weighed and then fixed in 10% neutral-buffered formalin. Tissues for microscopic examination (nasal turbinates, lungs, liver, and spleen) were embedded in paraffin, sectioned at 5  $\mu\text{m}$ , and stained with hematoxylin and eosin.

*In vitro metabolism of ferrocene.* Microsomes for *in vitro* studies of ferrocene metabolism were obtained from the cells of 14–20 week old unexposed male F344/N rats. The rats were killed by CO<sub>2</sub> asphyxiation. Nasal ethmo- and maxilloturbinates and the liver were removed and homogenized. Microsomes were separated and stored as described previously (Hadley and Dahl, 1982). Suspensions of microsomes, appropriate cofactors (Hadley and Dahl, 1982), and the radiolabeled ferrocene were incubated for 20 min at 37°C. Metabolism was stopped by adding 1 M KOH to raise the pH to  $\sim 11$ ; adding the KOH not only stopped the reaction, but also ionized phenolic metabolites to keep this species water soluble. After exhaustive hexane extractions of unmetabolized ferrocene, the aqueous portion of the reaction mixture was evaporated to dryness to remove, by sublimation, any small amounts of ferrocene that had not been extracted into the hexane. The residue was analyzed for radioactivity by liquid scintillation spectroscopy, and the protein content was determined with Lowry protein assay (Lowry *et al.*, 1951).

*Statistics.* The data from male and female animals were analyzed separately, using Bartlett's test for data homogeneity and Dunnett's test for the equality of mean values. The criterion for significance was set at  $p < 0.05$ .

## RESULTS

The overall mean ferrocene concentrations in each exposure chamber (2.5, 5, 10, 20, and 40 mg vapor/m<sup>3</sup>) during the 2-week, repeated study were within  $\pm 10\%$  of the target concentrations (Table 1). The relative standard deviation of the daily means was within 45%. The homogeneity (i.e., the variation among

TABLE 1

FERROCENE VAPOR CONCENTRATION SUMMARY 14-DAY, REPEATED INHALATION EXPOSURE OF RATS TO FERROCENE

Target concentration (mg/m <sup>3</sup> )	Actual concentration (mg/m <sup>3</sup> ) <sup>a</sup>	Percentage of target concentration
2.5	2.33 ± 1.05	93.20
5.0	5.29 ± 1.92	105.80
10	9.89 ± 2.12	98.90
20	20.02 ± 3.08	100.10
40	36.47 ± 7.81	91.18

<sup>a</sup> Mean ± SD (n = 78). Each ferrocene exposure concentration was shown to be statistically different from all other exposure concentrations at p < 0.05.

six position samples within each chamber) of the ferrocene vapor concentrations ranged from 5.7 to 17.6% among all exposure chambers.

No rats or mice died during the study, nor were any clinical signs of toxicity found during either the daily or the detailed observation times. Group mean body weights at the end of the study (Day 17) and mean weight gain over the exposure period are given in Tables 2 and 3 for rats and mice, respectively. Relative to control mice, male rats exposed to the highest concentration of ferrocene (40 mg/m<sup>3</sup>) had statistically significant decreases in terminal body weight and rate of weight gain during the exposures. No significant body-weight differences were seen among the groups of exposed and control female rats at the end of the study. Male mice exposed to the two highest concentrations of ferrocene (20 and 40 mg/m<sup>3</sup>) had significant decreases in terminal body weight and/or in the rate of weight gain, while female mice had decreases in terminal body weight and rate of weight gain at the three highest exposure levels (10, 20, and 40 mg/m<sup>3</sup>). No exposure-related gross lesions were found at necropsy in either rats or mice from any exposure group.

The only measured organ weight affected by ferrocene exposure in rats was that of the

liver. Group mean liver weights for male and female rats are given in Table 4. Male rats exposed to the highest concentration of ferrocene vapor had a statistically significant, although small, decrease in liver weights, compared to control animals. Statistically significant decreases in liver weight were not seen in female rats at any exposure concentration. Postexposure, group mean organ weights for male and female mice are given in Table 5. Male mice had a statistically significant decrease in liver and spleen weights after exposures to the two highest concentrations of ferrocene vapor, and a statistically significant increase in thymus weight after exposure to the highest concentration of ferrocene, when compared to control animals. Female mice had exposure-related decreases in brain, liver, spleen, and right kidney weights, relative to control female mice. The measured weights for other organs were not affected by ferrocene exposure (data not shown).

Because of the absence of any gross lesions in other organs, only lung, nasal turbinates,

TABLE 2

TERMINAL BODY WEIGHTS AND WEIGHT GAINS OF RATS AFTER THE 14-DAY, REPEATED INHALATION EXPOSURE STUDY OF FERROCENE

Exposure concentration (mg/m <sup>3</sup> )	Body weight (g) <sup>a</sup>	Percentage of control	Mean weight gain (g) <sup>a</sup>
Males			
0	172.26 ± 7.97	—	77.6 ± 2.7
2.5	180.02 ± 4.27	104.5	80.4 ± 4.1
5.0	175.70 ± 8.90	102.0	74.0 ± 11.5
10	152.48 ± 35.53	88.5	53.6 ± 33.5
20	173.44 ± 14.20	100.7	74.7 ± 9.5
40	163.98 ± 5.74 <sup>b</sup>	95.2	66.8 ± 2.8 <sup>b</sup>
Females			
0	123.16 ± 9.07	—	40.4 ± 4.3
2.5	123.04 ± 3.39	99.9	40.8 ± 3.1
5.0	119.92 ± 6.32	97.4	39.0 ± 2.2
10	121.06 ± 5.06	98.3	39.3 ± 6.1
20	119.20 ± 10.23	96.8	38.2 ± 4.6
40	117.96 ± 1.26	95.8	36.5 ± 5.4

<sup>a</sup> Means ± SD (n = 5).

<sup>b</sup> Statistically different from controls at p < 0.05 by Dunnett's test (data homogeneous by Bartlett's test).

TABLE 3

TERMINAL BODY WEIGHTS AND WEIGHT GAINS OF MICE AFTER THE 14-DAY, REPEATED INHALATION EXPOSURE STUDY OF FERROCENE

Exposure concentration (mg/m <sup>3</sup> )	Body weight (g) <sup>a</sup>	Percentage of control	Mean weight gain (g) <sup>a</sup>
<b>Males</b>			
0	26.06 ± 1.01	—	4.3 ± 1.1
2.5	25.66 ± 0.77	98.5	3.4 ± 1.0
5.0	25.30 ± 1.75	97.1	3.3 ± 0.8
10	25.24 ± 0.57	96.9	3.2 ± 1.1
20	24.42 ± 1.32	93.7	2.4 ± 1.1 <sup>b</sup>
40	23.58 ± 0.66 <sup>b</sup>	90.5	1.4 ± 0.4 <sup>b</sup>
<b>Females</b>			
0	23.02 ± 0.43	—	4.8 ± 0.8
2.5	22.34 ± 0.75	97.0	3.9 ± 0.4
5.0	22.06 ± 0.75	95.8	3.7 ± 0.7
10	21.62 ± 0.34 <sup>b</sup>	93.9	4.0 ± 1.0
20	20.64 ± 0.97 <sup>b</sup>	89.7	2.6 ± 1.2 <sup>b</sup>
40	19.98 ± 0.41 <sup>b</sup>	86.8	1.6 ± 0.5 <sup>b</sup>

<sup>a</sup> Means ± SD (n = 5).

<sup>b</sup> Statistically different from controls at p < 0.05 by Dunnett's test (data homogeneous by Bartlett's test).

liver, and spleen from each animal were examined microscopically. The only exposure-related histologic findings for either rats or mice were in the nasal olfactory epithelium. Neither species had lesions in the squamous

TABLE 4

LIVER WEIGHTS FOR RATS AFTER THE 14-DAY, REPEATED INHALATION EXPOSURE STUDY OF FERROCENE

Exposure concentration (mg/m <sup>3</sup> )	Liver weight (g) <sup>a</sup>	
	Male	Female
0	9.73 ± 0.86	5.45 ± 0.79
2.5	9.38 ± 0.47	5.54 ± 0.36
5.0	8.60 ± 0.89	5.25 ± 0.64
10	7.21 ± 2.09	5.10 ± 0.31
20	8.75 ± 1.29	5.08 ± 0.45
40	8.33 ± 0.46 <sup>b</sup>	5.06 ± 0.54

<sup>a</sup> Means ± SD (n = 5).

<sup>b</sup> Statistically different from controls at p < 0.05 by Dunnett's test (data homogeneous by Bartlett's test).

TABLE 5

ORGAN WEIGHTS FOR MICE AFTER THE 14-DAY, REPEATED INHALATION EXPOSURE STUDY OF FERROCENE

Exposure concentration (mg/m <sup>3</sup> )	Organ weight (g) <sup>a</sup>									
	Male					Female				
	Brain	Liver	Spleen	Thymus	Rt. kidney	Brain	Liver	Spleen	Thymus	Rt. Kidney
0	0.45 ± 0.1	1.51 ± 0.11	0.08 ± 0.02	0.040 ± 0.005	0.25 ± 0.01	0.46 ± 0.01	1.34 ± 0.11	0.09 ± 0.02	0.067 ± 0.015	0.19 ± 0.00
2.5	0.44 ± 0.02	1.58 ± 0.10	0.07 ± 0.00	0.047 ± 0.005	0.27 ± 0.02	0.44 ± 0.02 <sup>b</sup>	1.28 ± 0.03	0.09 ± 0.01	0.069 ± 0.007	0.19 ± 0.02
5.0	0.45 ± 0.01	1.42 ± 0.12	0.07 ± 0.01	0.050 ± 0.007	0.24 ± 0.03	0.44 ± 0.01	1.18 ± 0.16	0.08 ± 0.01	0.069 ± 0.012	0.16 ± 0.04
10	0.44 ± 0.02	1.37 ± 0.11	0.07 ± 0.01	0.050 ± 0.008	0.25 ± 0.02	0.45 ± 0.01	1.15 ± 0.08 <sup>b</sup>	0.07 ± 0.01 <sup>b</sup>	0.061 ± 0.014	0.18 ± 0.02
20	0.44 ± 0.02	1.24 ± 0.10 <sup>b</sup>	0.06 ± 0.01 <sup>b</sup>	0.051 ± 0.007	0.23 ± 0.02	0.43 ± 0.1 <sup>b</sup>	1.02 ± 0.10 <sup>b</sup>	0.06 ± 0.01 <sup>b</sup>	0.069 ± 0.012	0.16 ± 0.01
40	0.43 ± 0.02	1.27 ± 0.06 <sup>b</sup>	0.06 ± 0.01 <sup>b</sup>	0.053 ± 0.004 <sup>b</sup>	0.23 ± 0.02	0.42 ± 0.00 <sup>b</sup>	1.04 ± 0.07 <sup>b</sup>	0.07 ± 0.01 <sup>b</sup>	0.068 ± 0.013	0.15 ± 0.01 <sup>b</sup>

<sup>a</sup> Means ± SD (n = 5).

<sup>b</sup> Statistically different from controls at p < 0.05 by Dunnett's test (data homogeneous by Bartlett's test).

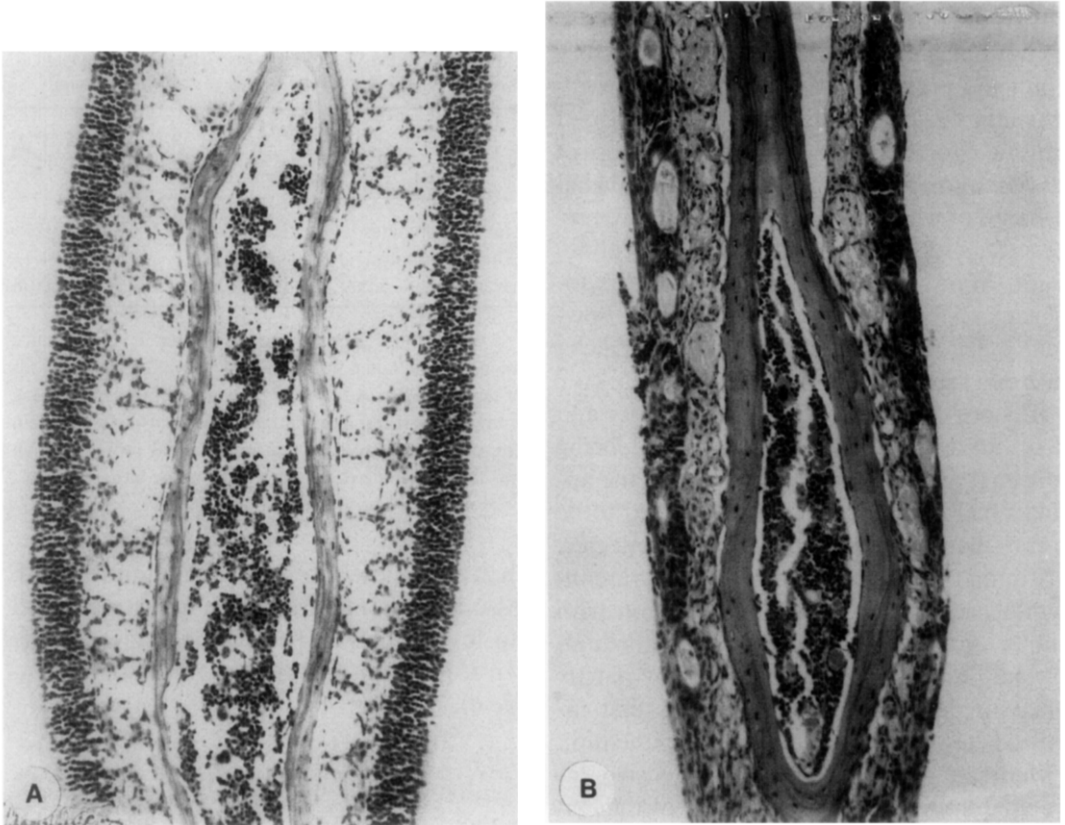


FIG. 1. Photomicrographs of nasal olfactory epithelium from control (A) and high level, ferrocene-exposed mice (B) ( $\times 135$ ). Note the marked attenuation, necrosis and accompanying inflammation of the nasal epithelium from the ferrocene-exposed animal (B). Similar nasal lesions were found in rats exposed to ferrocene vapor.

epithelium present at the entry to the nasal cavity. The respiratory epithelium was minimally affected; occasional necrosis of individual cells and occasional cells containing a granular, light-brown pigment within the cytoplasm were observed in this epithelium. These alterations were primarily centered in the nasal septum. The transitional epithelium that extends between the squamous and respiratory epithelial regions exhibited minimal necrosis and epithelial hyperplasia. Brown, cytoplasmic pigment, as described above, was also present within individual cells of this region. The severity of the lesions in the non-olfactory epithelium was exposure concentration-related, and both sexes and species were equally affected. However, the lesions were

minimal in degree, even in the group exposed to the highest concentration of ferrocene ( $40 \text{ mg/m}^3$ ).

The primary histopathological lesion for both rats and mice for both sexes and at all exposure levels was located in the olfactory epithelium (Fig. 1) and was most severe in the dorsal meatus. This lesion was classified as subacute, necrotizing inflammation and encompassed the features described below. The olfactory epithelium was characterized by patchy areas of degeneration and by necrosis of neuroepithelial and sustentacular cells. Many areas were markedly atrophic. Inflammatory cells, predominantly neutrophils, were present; however, they were not a prominent component of the reaction. The relative pau-

city of inflammatory cells that we observed is a common finding in lesions of the olfactory epithelium. The majority of the remaining epithelium was characterized by the presence of mitotic figures, rosette formation, and loss of basement membrane orientation of epithelial cells, all of which are indicative of marked regenerative attempts by the olfactory epithelium. Many epithelial cells contained cytoplasmic, light-brown, granular pigment. Bowman's glands were also characterized by patchy necrosis and atrophy.

Lesions were graded based on severity and distribution on a 1 to 5 scale, with 1 being minimal and 5 being severe. None of the animals had lesions that were considered to be more than moderate (grade 3) in degree. "Minimal" lesions were those having rare individual cell necrosis and degeneration with subtle atrophy of the olfactory epithelium. "Mild" lesions were characterized by patchy areas of degeneration and necrosis that involved less than 10% of the epithelium. "Moderate" lesions were more extensive, involving up to approximately 25% of the epithelium; an inflammatory response was more prominent in lesions of this severity.

The severity of the lesion in the olfactory epithelium was directly related to exposure dose. Lesions were most severe in the groups exposed to the highest ferrocene concentration (40 mg/m<sup>3</sup>) and were scored as moderate in degree. Lesions of a similar magnitude were also observed in the groups exposed to 20 mg/m<sup>3</sup> ferrocene. Lesions, minimal to mild in degree, were seen in the groups exposed to 5 and 10 mg/m<sup>3</sup> ferrocene. Lesions, minimal in degree, were seen in the group exposed to the lowest concentration of ferrocene, 2.5 mg/m<sup>3</sup>. We did not observe sex-related differences, and the severity of the lesion occurring after exposure to a given ferrocene concentration was about the same in rats and mice. No exposure-related microscopic lesions were found in either livers or spleens.

The *in vitro* studies of ferrocene metabolism showed that microsomes from the ethmoturbinates metabolized ferrocene more rapidly

TABLE 6

APPARENT KINETIC VALUES FOR THE METABOLISM OF FERROCENE BY NASAL AND LIVER MICROSOMES<sup>a</sup>

Tissue	$V_{\max}$ (nmol/min/g tissue)	$K_m$ ( $\mu$ M)	$\frac{V_{\max}}{K_m}$
Maxilloturbinates	3.9 $\pm$ 0.5	6 $\pm$ 2	0.7
Ethmoturbinates	8.8 $\pm$ 0.8	8 $\pm$ 2	1.1
Liver	1.2 $\pm$ 0.2	15 $\pm$ 2	0.08

<sup>a</sup> Values for  $V_{\max}$  and  $K_m$  and estimated 95% confidence intervals were taken from a plot of rate and concentrations of ferrocene derived by using 10 data points representing a range of ferrocene concentrations of 2 to 300  $\mu$ M. Protein concentration was 0.5–1 mg microsomal protein, which was then normalized to a per gram tissue basis.

than did those from the maxilloturbinates. Also, microsomes from both nasal tissues had up to 10 times higher activities towards the ferrocene than did the liver microsomes (Table 6).

## DISCUSSION

The localization of lesions in the olfactory mucosa is probably related to the high retention of ferrocene-derived iron in the nose (Dahl and Briner, 1980), which, in turn, is probably a result of the high metabolic capacity in the nose, particularly in the olfactory mucosa. Metabolism of ferrocene by cytochrome P450 probably proceeds according to the reactions shown in Fig. 2; ferrocene is metabolized to unstable hydroxyferrocene (Hanzlik *et al.*, 1978), which then decomposes, releasing free ferrous ion intracellularly. The ferrous ion can then catalyze lipid peroxida-

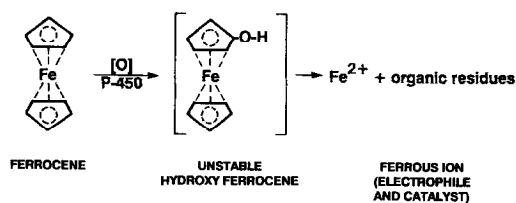


FIG. 2. Proposed metabolism of ferrocene.



tion (Dumelin and Tappel, 1977). This scenario could account for the lesions we observed. In addition to the high metabolic capacity of the olfactory epithelium, the greater sensitivity of this tissue to ferrocene toxicity as opposed to respiratory tract epithelium may also be due to a higher tissue dose and a lower rate of chemical clearance than respiratory epithelium. The olfactory epithelium lacks the ciliary clearance mechanism that is present in the respiratory epithelium. The olfactory epithelium also has fewer mucus-secreting cells and thus lacks the protective mucus layer that covers the respiratory epithelium.

Other inhalants that are metabolized to toxic products in the olfactory mucosa include dimethylnitrosamine (Reznik-Schüller, 1983), hexamethylphosphoramide (Lee and Trochimowicz, 1982), and 3-trifluoromethylpyridine (Gaskell *et al.*, 1988). The feature that is unique for ferrocene is the suggestion that metabolism leads to free intracellular ferrous ion, which produces toxicity by catalytic peroxidation. Experimental evidence for such a mechanism of toxicity for ferrocene has been provided by Eidson *et al.* (1989).

Other metabolically active sites, including the lung bronchioles, in which Clara cells are located, and the liver, were not affected by inhaled ferrocene. This may reflect either less rapid ferrocene metabolism (as is the case for rat liver) or less penetration of ferrocene into those regions (Dahl and Briner, 1980).

Exposure-related decreases in organ weights were seen for liver in rats, and in mice, liver, spleen, kidney, and brain weights also decreased, while thymus weights increased. While these results suggest that the named tissues may be target organs for toxicity resulting from long-term exposures to ferrocene, it is more likely that these changes were secondary effects brought on by the nasal lesions. However, in the case of liver, which, like olfactory epithelium, is highly active in xenobiotic metabolism, it is possible that primary toxic effects may result if ferrocene reaches this organ. Also, the differences between the two species in the effects of ferrocene exposure on body

and organ weights indicate that mice may be more susceptible to ferrocene toxicity than are rats, and that in longer-term exposure, female mice may be more sensitive to the toxic effects of ferrocene than are male mice. The results from these studies were used to select the exposure concentrations for a 13-week subchronic inhalation exposure study. These 13-week subchronic inhalation exposures, using concentrations of 3.0, 10, and 30 mg/m<sup>3</sup> ferrocene, are currently in progress to determine the cumulative toxicity of ferrocene.

#### ACKNOWLEDGMENTS

The authors are indebted to a number of individuals on the technical staff for their assistance on this project. This research was conducted in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care. This research was sponsored by the National Institute of Environmental Health Sciences/National Toxicology Program under Interagency Agreement Y01-ES-70157 and the Office of Health and Environmental Research of the U.S. Department of Energy under Contract DE-AC04-76EV01013.

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