Metabolism of Mercury, Administered as Methylmercuric Chloride or Mercuric Chloride, by Lactating Ruminants

Jerry L. Sell* and Kenneth L. Davison

A milk goat and a milk cow were given single tracer doses of $CH_3{}^{203}HgCl$ by intraruminal injection. A high proportion of $CH_3{}^{203}HgCl$ was absorbed with less than 20% of the dose appearing in feces within 72 hr of treatment. Cumulative secretion of ^{203}Hg in goat's milk over a 13-day period was 0.28% of the dose. Radioactivity was not detected in cow's milk. The goat and cow were killed 13 and 7 days, respectively, after dosing. Distribution of ^{203}Hg among tissues was similar for the two species. Highest concentrations occurred in kidney, liver, and muscle. In a second experiment, apparent absorption was 80% of the dose when a goat was given $CH_3{}^{203}HgCl$ daily for 9 days. A goat given equivalent amounts of Hg in the form of $^{203}HgCl_2$ absorbed less than 30% of the dose. Secretion of ^{203}Hg in milk during a 36-day period following start of treatment totalled 0.22 and 1.12% of the dose for the $^{203}HgCl_2$ and $CH_3{}^{203}Hg$-treated goats, respectively. Half-times of retention of ^{203}Hg by goats given $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ were 78 and 22 days, respectively.

Incidents of accidental poisoning with mercury (Hg) (Bakir et al., 1973; Curley et al., 1972; Kutsuna, 1968) and observations of relatively high levels of Hg in fish (Fimreite et al., 1971; Wobeser et al., 1970) and in wild birds (Fimreite et al., 1970; Swanson et al., 1971) created much concern about possible Hg contamination of foodstuffs. Recently, data obtained from surveys showed that Hg concentrations were low in foods originating from commercial agriculture (Sell et al., 1975; Somers, 1971; Tanner et al., 1972).

Of the animal products analyzed by Sell et al. (1975), fresh whole milk contained the lowest concentration of Hg, less than one part per billion (ppb). Information gathered on animal feeds showed that forages consumed by dairy cattle often contained relatively high Hg levels—100 to 350 ppb (Sell and Deitz, 1973). Thus, it appeared that only a small proportion of Hg ingested by lactating cows was transferred to milk. Information on this point is meager. Howe et al. (1972) and Potter et al. (1972) presented evidence that little radioactive Hg appeared in goat's and cow's milk following the oral administration of single doses of ^{203}Hg-labeled mercuric chloride. Neathery et al. (1974) reported similar observations following treatment of cows with a single oral dose of ^{203}Hg-labeled methylmercuric chloride.

In view of the limited amount of information available, research was conducted to determine the magnitude of secretion of Hg administered orally as mercuric chloride ($HgCl_2$) or as methylmercuric chloride (CH_3HgCl) in milk of lactating ruminants. In addition, data were obtained concerning patterns of excretion of Hg in urine and feces, and the distribution of Hg among body tissues.

EXPERIMENTAL SECTION

Reagents. Reagent grade mercuric chloride and methylmercuric chloride were obtained from Fisher Scientific Co. (Fair Lawn, N.J.) and Alfa Inorganics (Ventron, Beverly, Mass.), respectively. [^{203}Hg]Methylmercuric chloride (specific activity, 130 mCi/g; radiopurity, in excess of 95%) was purchased from Amersham-Searle Corp. (Arlington Heights, Ill.). [^{203}Hg]Mercuric chloride (specific activity, 20 Ci/g; radiopurity, in excess of 99%) was obtained from New England Nuclear (Boston, Mass.).

Animal Science Department, North Dakota State University (J.L.S.), and U.S. Department of Agriculture, Agricultural Research Service, Metabolism and Radiation Research Laboratory, Fargo, North Dakota 58102.

Apparatus. Radioactivity was determined with a Model 1085 deep-well γ counter equipped with a NaI (thallium activated) crystal (Nuclear-Chicago, Chicago, Ill.). A Model 305 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) was used in the determination of mercury. The carcasses of animals were ground with a Model 801 GP 25 grinder (Autio Co., Astoria, Ore.), and the grindings were homogenized with a Model H-600 mixer (Hobart Manufacturing Co., Troy, Ohio).

Procedure. *Experiment 1.* The main objective of experiment 1 was to compare a lactating goat with a lactating cow with respect to the metabolism of Hg when this element was given as CH_3HgCl. A 4-year-old Nubian goat in the late stage of lactation and a 6-year-old Guernsey cow in the third month of lactation were used. Both animals were kept in metabolism cages for the quantitative collection of feces. Catheters were used in each animal to collect urine. Alfalfa hay was given ad libitum, and pellets containing grain and minerals were supplied in quantities appropriate for level of milk production. The goat weighed 70 kg at the start of the experiment, while the cow weighed 680 kg.

Following a 7-day adaptation period, the goat was given 100 μCi of radioactivity in the form of $CH_3{}^{203}HgCl$ (dissolved in 95% ethanol) by intraruminal injection. The cow was given 500 μCi of $CH_3{}^{203}HgCl$ by the same method. Blood samples were taken from each animal by puncture of the jugular vein at 2, 4, 8, 12, and 24 hr. post-treatment. Subsequently, blood samples were taken immediately after morning and evening milkings each day for the remainder of the experiment. Heparin was used to prevent coagulation of blood.

Total milk, urine, and feces produced by each animal were weighed and sampled twice daily. All samples were analyzed for radioactivity. The cow and goat were killed 7 and 13 days, respectively, following dosing. Selected tissues were excised, weighed, and blended prior to sampling for determination of radioactivity. The remainder of the goat carcass, including viscera, was ground and homogenized prior to sampling. The carcass of the cow, excluding the excised tissues, leg bones, and skull, was ground and homogenized for sampling.

Experiment 2. Experiment 2 was conducted to extend observations on CH_3HgCl metabolism and to obtain comparative data on the metabolism of Hg given as $HgCl_2$. Two Saanen goats, each 2 years old and in early lactation, were kept in metabolism cages throughout the experiment. After a 7-day adaptation period, goat A, which weighed 44 kg, was given 22 mg of Hg in the form of CH_3HgCl (0.5 mg of Hg/kg body weight) and 80 μCi of [^{203}Hg]CH_3HgCl, both in a single gelatin capsule administered orally. Goat B, which

Table I. Distribution of ^{203}Hg among Tissues of the Cow and Goat 7 and 13 Days after Intraruminal Injection of $CH_3{}^{203}HgCl$; Experiment 1

	dpm per g		% of total dose[c]	
	Goat[a]	Cow[b]	Goat	Cow
Liver	20,040	7680	6.57	6.35
Skin and hair	1,760	340	4.89	1.65
Kidney	17,120	8280	1.24	1.24
Lungs	3,740	1560	0.60	0.68
Heart	2,660	1960	0.32	0.62
Brain	2,920	1220	0.12	0.054
Pancreas	1,460	1240	0.06	0.065
Adrenals	2,120	840	0.003	0.0003
Muscle (l. dorsi)	7,660	3460		
Spinal cord	1,680	460		
Bile (per ml)	420	80		
Adipose tissue	160	60		

[a] Killed 13 days after dosing. [b] Killed 7 days after dosing. [c] The total recoveries of the administered dose (cow, 500 μCi, and goat, 100 μCi), including carcass, viscera, excised tissues, milk, urine, and feces, were 97.9 and 96.5% for the goat and cow, respectively.

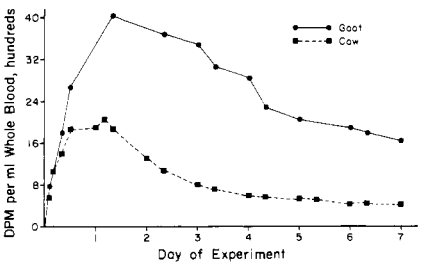

Figure 1. Radioactivity in whole blood of cow and goat after a single, intraruminal injection of $CH_3{}^{203}HgCl$; experiment 1.

weighed 42 kg, was given equivalent amounts of Hg and radioactivity in the form of $HgCl_2$ and $[^{203}Hg]HgCl_2$, respectively. Treatment of goat A with CH_3HgCl and of goat B with $HgCl_2$ continued for 8 more days, the daily oral doses being 0.5 mg of Hg/kg body weight and 40 μCi of ^{203}Hg radioactivity.

Blood samples were taken from each goat at 2, 4, 8, 12, and 24 hr after start of treatment, and once daily thereafter. The cells and plasma were separated by centrifugation, and the radioactivity of each fraction determined. Milk, urine, and feces were collected quantitatively twice daily and assayed for radioactivity. In this experiment, urine was collected in stainless steel pans and funnels attached to the metabolism cages (Robbins and Bakke, 1967). Collections were terminated after 36 days.

Radioactivity of all samples was measured using a deep-well γ counter. The volume and geometry of the samples were standardized to 1 cm^3 and compared with appropriate standards. All data on radioactivity were corrected for natural decay.

Selected samples of milk produced by goats A and B were also analyzed for total Hg by the method of Deitz et al. (1973). This procedure involved wet oxidation with a nitric–sulfuric acid mixture followed by cold vapor atomic absorption spectrophotometry.

RESULTS

Experiment 1. Two hours after administering $CH_3{}^{203}HgCl$, radioactivity was readily detectable in blood of the goat and cow (Figure 1). Radioactivity increased rapidly through 12 hr post-treatment with the cow, then plateaued for a short time, and declined during the remainder of the experiment. The maximum level of radioactivity attained was higher in goat blood than in that of the cow. These observations probably do not indicate a difference between species in absorption or metabolism of CH_3HgCl. The cow had a nearly tenfold greater body mass as compared with the goat (680 kg vs. 70 kg) and was given only five times as much radioactivity. Consequently, the doses were 1.43 and 0.74 μCi per kg body weight for the goat and cow, respectively, and a lower level of radioactivity in blood might have been expected for the cow.

Table II. Cumulative Elimination of Radioactivity in Milk, Feces, and Urine of a Cow and a Goat Given $CH_3{}^{203}HgCl$ by Intraruminal Injection; Experiment 1

Day after start of expt[a]	% of total dose					
	Goat			Cow		
	Milk	Feces	Urine	Milk	Feces	Urine
1	N.D.[b]	0.55	0.12	N.D.	4.62	0.18
3	0.08	16.56	0.63	N.D.	18.11	0.75
5	0.14	21.72	0.90	N.D.	22.81	1.04
7	0.19	24.61	1.11	N.D.	25.32	1.28
13	0.28	31.18	1.45			

[a] The goat and cow were given 100 and 500 μCi of ^{203}Hg, respectively, as a single dose. [b] N.D. indicates radioactivity was not detectable.

Similar patterns of distribution of radioactivity were observed among tissues of the cow and the goat (Table I) when the animals were killed 7 and 13 days after dosing, respectively. Highest levels of radioactivity were found in the liver and kidneys. Relatively high levels were also detected in lungs, heart, and brain. The liver contained the highest percentage of dose followed by the skin plus hair and the kidney.

The samples of longissimus dorsi had a relatively high concentration of radioactivity while the adipose tissue taken from the abdominal cavity and from the area covering the l. dorsi was quite low. Carcasses of the goat and cow, including viscera but excluding excised organs and tissues, contained 52.4 and 56.2% of the total dose, respectively. No attempt was made to determine the percentages of dose in muscle, fat, etc. of the carcasses. However, the relatively high concentration of radioactivity observed in muscle together with the fact that muscle undoubtedly comprised a sizable part of carcass weight suggests that a large portion of the Hg administered was retained in muscle tissue.

Low concentrations of radioactivity were found in the milk of the goat beginning at 32 hr after dosing (Table II). No radioactivity was detected in cow's milk during the experiment. This discrepancy between cow and goat was probably related to differences in body mass mentioned previously as well as to difference in volume of milk produced vs. amount of radioactivity administered. The cow produced 20 kg of milk per day as compared with about 1 kg per day for the goat. Only a small proportion of total radioactivity given the goat was secreted in milk during the 13-day trial, the cumulative secretion being 0.28% of the total dose (Table II).

On the basis of fecal excretion, the apparent absorption of ^{203}Hg by both animals exceeded 80% of the dose within 3 days of dosing (Table II). The data of Table II also show that, after absorption, the elimination of ^{203}Hg from the body was not rapid, and that the major pathway of excretion was the feces.

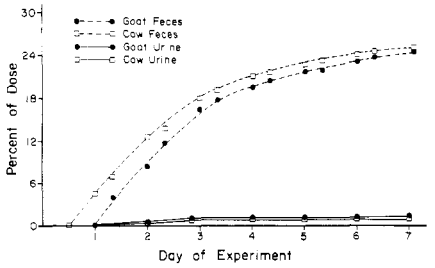

Figure 2. Cumulative excretion of ^{203}Hg in feces and urine of cow and goat after a single, intraruminal injection of $CH_3{}^{203}HgCl$; experiment 1.

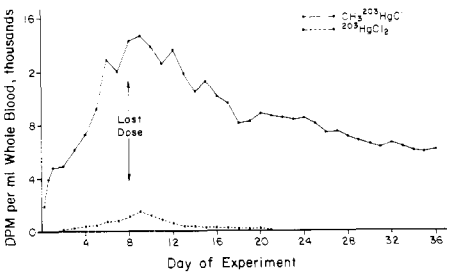

Figure 3. Radioactivity in whole blood of goats given $CH_3{}^{203}HgCl$ or $^{203}HgCl_2$ orally for 9 successive days; experiment 2.

Comparatively, apparent absorption and rate of excretion of ^{203}Hg were similar for the cow and goat. This was especially apparent when cumulative excretions in feces and urine were depicted graphically (Figure 2).

The total radioactivity recovered from the milk, urine, feces, and body tissues of the cow and goat accounted for 96.5 and 97.9% of the total dose, respectively.

Experiment 2. As was observed in experiment 1, radioactivity appeared in blood of goat A treated with $CH_3{}^{203}HgCl$ within 2 hr of start of treatment (Figure 3) and increased rapidly as treatment continued. In contrast, ^{203}Hg was not detected in blood of goat B until 2 days after the first oral dose of $HgCl_2$ was given. Radioactivity in blood of the $HgCl_2$-treated goat increased slowly with continued treatment, peaked at day 9 of the trial, and decreased to essentially nondetectable levels by day 21. Radioactivity also reached a maximum at day 9 for the CH_3HgCl-treated goat and declined slowly thereafter.

The maximum radioactivity attained in blood of the $HgCl_2$-treated goat B was about 1200 dpm/ml while that for goat A, given CH_3HgCl, was nearly 15,000 dpm/ml. Since the amounts of radioactivity and total Hg given daily to each goat were equivalent, the difference in blood radioactivity probably reflects differences in both the rate and magnitude of absorption of Hg from the two chemical forms.

There was also a marked difference between treatments with respect to radioactivity in the packed cells and plasma. More than 90% of the total ^{203}Hg in blood of the goat given CH_3HgCl was in the packed cells beginning with the blood sample taken at 2 hr after start of treatment. This distribution of radioactivity in the two blood fractions persisted throughout the experiment. However, only 45–50% of the ^{203}Hg in blood of the $HgCl_2$-treated goat was in the packed cells from day 2 through day 9 of the experiment. Subsequently, the proportion of radioactivity in packed cells increased steadily with time after cessation of $HgCl_2$ treatment, and by day 18 comprised 70% of the blood's radioactivity.

Radioactivity was found in milk of both goats 1 day after start of the experiment (Figure 4). The concentration of

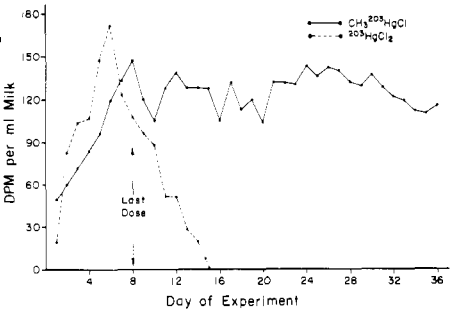

Figure 4. Radioactivity in milk produced by goats given $CH_3{}^{203}HgCl$ or $^{203}HgCl_2$ orally for 9 successive days; experiment 2.

^{203}Hg in milk of the $HgCl_2$-treated goat B exceeded that of goat A given CH_3HgCl, beginning at day 2 and persisting through day 6 of the experiment. This observation was particularly interesting in view of the relatively low blood ^{203}Hg values for goat B. When at the highest level, the ^{203}Hg concentration in milk of the $HgCl_2$-treated goat was $\frac{1}{5}$ that of whole blood and was $\frac{1}{2}$ that of plasma. In contrast, the ^{203}Hg level in milk of the goat given CH_3HgCl was $\frac{1}{100}$ that of whole blood and $\frac{1}{5}$ that of plasma when at its highest concentration.

Goats A and B produced essentially the same volumes of milk (2 to 2.1 kg daily) while on experiment. Thus, this factor would not have affected the level of ^{203}Hg in milk. It appears that concentration of ^{203}Hg in milk was related to the manner with which the two forms of Hg were handled metabolically, and was more closely related to plasma levels of ^{203}Hg than to ^{203}Hg of whole blood or cells.

The data depicted in Figure 4 also show that a decline of ^{203}Hg in milk of the $HgCl_2$-treated goat began prior to cessation of treatment. No explanation is available for this occurrence but the downward trend continued through day 15 of the trial (7 days after treatment stopped), at which time no radioactivity was detected.

The radioactivity of milk produced by goat A increased gradually as CH_3HgCl treatment continued and then remained at a fairly constant level through day 36, 28 days after treatment stopped. The data are not shown but subsequent sampling showed that radioactivity in the milk of goat A had decreased to about 28 dpm 60 days after the experiment began.

The concentration of total Hg in milk of both goats paralleled changes in ^{203}Hg with time on experiment. The highest concentrations measured were 61 ppb at day 6 for the $HgCl_2$-treated goat and 45 ppb at day 8 for the goat given CH_3HgCl. The specific activities of milk Hg reached a maximum within 1 day after start of treatment for the CH_3HgCl-treated goat and within 2 days for the $HgCl_2$-treated goat. Specific activities remained constant through day 18.

The data on cumulative secretion of ^{203}Hg in milk (Table III) show that only a small proportion of the total Hg administered was transferred into milk, irrespective of source. The goat given CH_3HgCl secreted more total radioactivity into milk than did the goat given $HgCl_2$, probably due to the greater degree of absorption of Hg from CH_3HgCl and the persistence of high levels of radioactivity in the blood of the CH_3HgCl-treated goat.

Additional research with a limited number of milk samples from each goat revealed that none of the ^{203}Hg was in the lipid fraction (lipids isolated from a chloroform–methanol extract of lyophilized milk). All radioactivity was found in the solids-not-lipid fraction of milk.

As was observed in experiment 1, a high proportion of ^{203}Hg given as CH_3HgCl was apparently absorbed (Table III). Aftter 8 days of continuous administration of CH_3HgCl, less than 20% of the total dose appeared in feces.

Table III. Cumulative Elimination of Radioactivity in Milk, Feces, and Urine of Goats Given CH_3HgCl or $HgCl_2$ for 9 Successive Days; Experiment 2

Day after start of treatment[a]	% of total dose					
	Goat A, given $CH_3{}^{203}HgCl$			Goat B, given $^{203}HgCl_2$		
	Milk	Feces	Urine	Milk	Feces	Urine
1	0.06	8.78	0.29	0.02	3.84	0.02
3	0.16	16.88	0.50	0.09	53.79	0.10
5	0.20	18.91	0.69	0.14	55.27	0.24
9	0.29	19.77	1.03	0.18	74.93	0.37
13	0.42	29.28	1.44	0.21	86.47	0.61
18	0.58	36.47	2.26	0.22	87.03	0.93
36	1.12	60.00	4.39	0.22	88.02	1.46

[a] Goats A and B were given cumulative doses of 400 μCi of ^{203}Hg and 198 mg of Hg in the form of CH_3HgCl and $HgCl_2$, respectively.

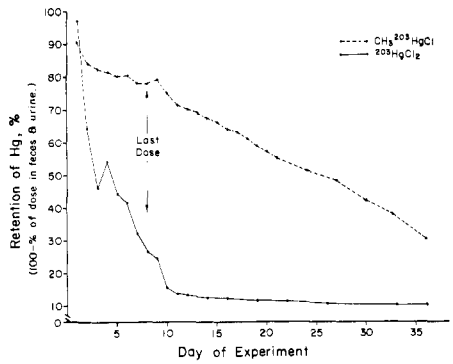

Figure 5. Retention of ^{203}Hg by goats given $CH_3{}^{203}HgCl$ or $^{203}HgCl_2$ orally for 9 successive days; experiment 2.

Presumably, a portion of this fecal radioactivity was ^{203}Hg re-entering the digestive tract following absorption.

The apparent absorption of Hg from $HgCl_2$ was relatively low (Table III). Approximately 75% of the total dose was excreted in feces shortly after treatment was stopped. After 13 days of the experiment, more than 86% of the ^{203}Hg given as $HgCl_2$ appeared in feces while, in contrast, about 30% of the total ^{203}Hg administered as $CH_3{}^{203}HgCl$ was excreted in feces during the same time interval.

The greater absorption of Hg from CH_3HgCl than from $HgCl_2$ was also reflected by cumulative excretion of radioactivity in urine (Table III). By the time the experiment was terminated at day 36, about 4.4% of the ^{203}Hg given as CH_3HgCl was found in urine as compared with 1.46% of the ^{203}Hg given as $HgCl_2$.

Utilizing the information on urinary and fecal excretion of ^{203}Hg and ignoring ^{203}Hg in milk, the apparent retention of radioactivity by goats was calculated (100 − %) of dose in urine and feces) for various times of the experiment. These data are illustrated in Figure 5. In the case of the goat given $HgCl_2$, there was a rapid excretion of ^{203}Hg, primarily in feces, through day 10 of the experiment (2 days after treatment stopped). Subsequently, ^{203}Hg excretion proceeded at a slow, steady rate. The rate of excretion from days 10 through 36 indicated a half-life of retention of about 78 days.

The excretion rate of ^{203}Hg by the CH_3HgCl-treated goat was relatively constant throughout the experiment and appeared to be related to body burden of ^{203}Hg at a specific time. The calculated half-life of ^{203}Hg (derived from CH_3HgCl) in the body was 22 days.

DISCUSSION

The results of experiments 1 and 2 showed that a high proportion of Hg given orally or by intraruminal injection as CH_3HgCl was rapidly absorbed by a cow and by goats. A rapid and relatively high rate of absorption of ^{203}Hg was observed whether $CH_3{}^{203}HgCl$ was given as a single tracer dose or as successive daily doses in combination with nonradioactive CH_3HgCl. Aberg et al. (1969) reported that nearly all Hg given orally as methylmercury was absorbed from the digestive tract of man and mice. Also, Friberg and Vostal (1972) cited evidence that very high proportions of Hg were absorbed when administered in methylmercury form. Neathery et al. (1974) observed that the apparent absorption of a single oral dose of $CH_3{}^{203}HgCl$ by cows was high and that 59% of the dose was retained in the body 14 days after dosing.

Clarkson (1971) reported that less than 2% of the Hg given as $HgCl_2$ was absorbed by mice. Similarly, Howe et al. (1972) and Potter et al. (1972) found that the $HgCl_2$ form of Hg was very poorly absorbed by goats and cows, respectively. Data obtained in the research reported here also showed that the goat apparently absorbed less than 20% of the Hg given as $HgCl_2$ in nine successive daily oral doses.

In addition to showing that the goat and cow resemble each other with respect to the rate of magnitude of CH_3HgCl absorption, the results of experiment 1 showed that distribution of Hg among body tissues was similar for the two species. Highest Hg concentrations were found in liver and kidney, although other tissues, including muscle and brain, had notable levels of Hg. Previously, Swensson and Ulfvarson (1968) reported that Hg from CH_3HgCl was widely distributed among body tissues. More recently, Ansari et al. (1972) working with calves and Neathery et al. (1974) using cows observed that, even though highest Hg levels were found in liver and kidneys, fairly high Hg levels were present in muscle. In fact, Neathery et al. (1974) estimated that about 72% of the total ^{203}Hg in the body of cows given $CH_3{}^{203}HgCl$ was in muscle.

A marked difference in the proportion of blood Hg present in plasma and cells was observed when CH_3HgCl and $HgCl_2$ were given to goats during a 9-day treatment period. Approximately 90% of Hg in blood of the CH_3HgCl-treated goat was found in packed cells while the packed cells of blood from the $HgCl_2$-treated goat contained less than 50% of the total Hg. Subsequent to cessation of Hg treatment, Hg content of the $HgCl_2$-treated goat's packed cells increased gradually, reaching 70% of the total blood Hg by 10 days after treatment was stopped.

Lundgren et al. (1967) and Suzuki et al. (1970) reported that the red blood cells (RBC) of humans contained 20 times as much Hg as plasma when CH_3HgCl was administered, but that when $HgCl_2$ was used, Hg level in the RBC was twice that of plasma. Potter et al. (1972) reported similar findings when calves were given $HgCl_2$ intravenously.

Olsen et al. (1973) added CH_3HgCl or $HgCl_2$ to trout

blood in vitro and found that RBC bound more than 90% of the CH_3HgCl while only 9% of the $HgCl_2$ was bound by RBC during the same time interval. Olsen et al. (1973) also reported that an increasing amount of $HgCl_2$ became associated with RBC as in vitro incubation time increased. Friberg and Vostal (1972) referred to the gradual increase in proportion of blood Hg found in RBC as time after $HgCl_2$ dosing progressed. Our results with the $HgCl_2$-treated goat corroborate these observations.

Data on the transfer of Hg into milk of animals are meager. Trenholm et al. (1971) observed levels of Hg in milk of guinea pigs treated with methyl mercury that were about 5 to 10% of the concentration found in blood. Potter et al. (1972) reported a cumulative secretion of 0.01% of a single oral dose of Hg from $HgCl_2$ in milk of cows during a 6-day post-treatment period. Similarly, Howe et al. (1972) found that goats given $^{203}HgCl_2$ orally secreted less than 0.02% of the dose in milk during a 16-day post-treatment period. Neathery et al. (1974) presented data showing that only 0.17% of the Hg given orally as CH_3HgCl was found in milk over a 14-day interval after treatment.

The results of experiment 1 of the current research resemble those of Neathery et al. (1974). Following a single oral dose of CH_3HgCl, about 0.28% of the dose was found in goat milk during a 13-day post-treatment period while no Hg was found in cow's milk. When daily doses of CH_3HgCl or $HgCl_2$ were given for 9 days (experiment 2), 0.42% of the ^{203}Hg from $CH_3{}^{203}HgCl$ was transferred to milk produced over 13 days while only 0.21% of the $^{203}HgCl_2$ was found in milk.

From days 13 through 36 of experiment 2, essentially no measurable secretion of Hg in milk of the $HgCl_2$-treated goat occurred while, in the case of the goat given CH_3HgCl, Hg continued to appear in milk. This difference appeared to be related to blood Hg levels. Mercury in blood of the CH_3HgCl-treated goat remained at a relatively high level following cessation of treatment while, in contrast, blood Hg of the goat given $HgCl_2$ declined to a nondetectable level by day 21 of the experiment.

In biological systems, Hg (especially Hg from methylmercury) tends to become associated with proteins (Clarkson, 1972; Sell et al., 1974). This appears to also be probable for Hg in milk since all Hg present in milk produced by goats treated with CH_3HgCl or $HgCl_2$ was found in the nonlipid solids.

The data on excretion of Hg in feces and urine by goats showed that the major pathway for elimination of Hg from the body was in feces, irrespective of the Hg source. This has been demonstrated with several species of animal (Friberg and Vostal, 1972). However, the rate of fecal excretion of Hg depends in part on the form of Hg administered. Stake et al. (1974) observed that 28.3% of the ^{203}Hg given as $^{203}HgCl_2$ to calves by intravenous injection (iv) was excreted in feces during a 7-day post-treatment period. Only 8.1% of a dose of $CH_3{}^{203}HgCl$ administered iv appeared in feces during the same time interval.

Clarkson (1972) concluded in a review that Hg derived from methylmercury was excreted at a rate directly related to the simultaneous body burden. Also, the half-times of Hg retention were the same whether mice were given methylmercury as a single oral dose or were fed food which contained methylmercury (Clarkson, 1972).

Mercury originating from methylmercury is excreted according to first-order kinetics, and estimates of the half-times of retention of methylmercury vary from 8 days for mice to 70 days for man and other primates. In the current study, the half-time of Hg retention, based on Hg excretion in feces and urine by the CH_3HgCl-treated goat, was 22 days.

In contrast to methylmercury, the excretion pattern for Hg given as $HgCl_2$ has been found to be quite complex (Rothstein and Hayes, 1960). Rats treated with $HgCl_2$ excreted Hg in three phases. Initially, there was a rapid phase which lasted a few days and accounted for about 35% of the dose. This was followed by a slower phase which had a half-time of retention of 30 days and involved 50% of the dose. Excretion of Hg from $HgCl_2$ was very slow during the third phase, accounting for 15% of the dose and having a half-time of retention of about 100 days.

The pattern of Hg excretion in feces and urine of the goat given $HgCl_2$ in the present study was at least biphasic. The initial phase was apparent during the 8-day treatment period and for 2 days thereafter. Excretion of Hg during this phase was rapid and probably consisted largely of unabsorbed Hg. A second phase was evident beginning on day 11 of the trial and persisting through the 36-day experiment. The rate of Hg excretion during this latter phase was very slow and appeared to follow first-order kinetics. The half-time of retention during the second phase for the $HgCl_2$-treated goat was 78 days, a value similar to that reported by Rothstein and Hayes (1960) for the slowest phase of Hg excretion by rats given $HgCl_2$.

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Parathion Residues on Citrus Foliage. Decay and Composition as Related to Worker Hazard

Robert C. Spear,* William J. Popendorf, John T. Leffingwell, and David Jenkins

The decay of ethylparathion on citrus foliage was followed for 16 days post-application in 32 groves in central California. Paraoxon comprised a significant fraction of the foliar residue in all groves. In 31 fields paraoxon decayed more slowly than parathion. The combined residues of parathion and paraoxon degrade such that the hazard to workers decreases with time.

Foliar residues of organophosphate pesticides have been implicated in sporadic incidents of illness among agricultural fieldworkers in the western United States over the past 25 years (Quinby and Lemmon, 1958). It has been uncertain whether this problem is restricted to the semiarid agricultural regions of the U.S. or, given the world-wide pattern of organophosphate pesticide use, whether incidents occur and are not reported elsewhere. Recent information suggests that poisonings attributed to O–P residues have occurred in El Salvador within the last several years (Davies et al., 1975). In the United States, concern over this occupational health problem caused the establishment of regulations governing the entry of workers into treated fields (California Department of Food and Agriculture, 1971; *Fed. Regist.*, 1974). These regulations define the allowable time intervals between application of the pesticide and entry into the field or grove if work involves substantial foliar contact and appear to be a practical means of controlling exposures in conditions where medical surveillance is impractical.

The re-entry concept is based on an assumption that the potential for intoxication from foliar residues is a decreasing function of time. As the concept has been applied, it is further assumed that the decay process varies so little that a single re-entry interval can be equitably applied to a given pesticide–crop combination regardless of regional or climatological differences. However, the relative infrequency of reported organophosphate poisoning incidents suggests that an unusual combination of conditions must exist to cause overt intoxication. In the case of parathion such rare events may result from prolonged persistence of the pesticide residues or from the formation and persistence of a more toxic oxygen analog (Milby et al., 1964).

We have investigated the validity of the assumptions underlying the re-entry concept in a climatologically homogeneous region and attempted to discover a relationship between application variables and unusual persistence of residues. The focus of attention was on the degradation rate of the dislodgeable residues of parathion on citrus foliage, since this pesticide–crop combination has been implicated in a number of poisoning incidents. Dislodgeable residues were determined using the technique of Gunther et al. (1973).

Department of Biomedical and Environmental Health Sciences, School of Public Health (R.C.S., W.J.P., J.T.L.), and the Department of Civil Engineering and Sanitary Engineering Research Laboratory (D.J.), University of California, Berkeley, California 94720.

EXPERIMENTAL SECTION

Grove Selection and Sampling Procedure. Sixteen Valencia orange groves and sixteen groves of navel oranges in Tulare County, Calif., were studied. All 32 groves received commercial applications of ethylparathion between May 15 and 24, 1973. Although 27 of the 32 applications were wettable powder formulations, the diversity of additive chemicals, irrigation practices, and rates of application otherwise reflected commercial practice in the region. The local weather during the study is summarized in Figure 1.

Sampling in each grove commenced 2 days after application in order to avoid the rapid initial loss of residue (Ebeling, 1963). A random sample of 15 trees was chosen from along a diagonal line through each grove. Subsequent samples were taken on days 9 and 16 post-application from the same trees in order to minimize tree-to-tree variability. Each sample consisted of a total of 60 leaf disks, each 3 cm in diameter, taken from the 15 trees according to the procedures of Gunther et al. (1973). The sample bottles were frozen immediately and returned to the laboratory for extraction and analysis for both parathion and paraoxon.

Materials and Apparatus. Gas chromatographic analysis was performed on a Varian 1520 equipped with alkali flame ionization detectors. Columns were 6 ft × 2 mm glass packed with either 10% DC 200 or an equal mixture of 10% DC 200 and 15% QF-1 on 100–120 mesh Chromosorb W (HP) and were run at 220°. Detector and on-column injector temperatures were 245 and 235°, respectively. Carrier gas was nitrogen (20 ml/min) and flame parameters were 10 ml/min for hydrogen and 75 ml/min for air. The minimum detectable level for parathion ranged from 1–5 pg while for paraoxon it fell between 3 and 10 pg. The identity of paraoxon was confirmed by gas chromatography–mass spectrometry. The GC–MS was a Finnigan 1015D with a Systems Industries 150 control system and a chemical ionization source. Carrier gas was methane and the column was 6 ft × 4 mm glass packed with 3% OV-17 on 60–80 mesh Gas-Chrom Q, run at 205°. The parathion and paraoxon standards were obtained from Chem Service Inc., West Chester, Pa. Extractions were made with commercial pesticide grade solvents.