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# Hexachlorobenzene Contamination in Laboratory Monkey Chow

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The presence of hexachlorobenzene contamination in some samples of Purina monkey chow was established on the basis of gas chromatographic and mass spectrometric evidence. Among some 30 batches of Purina monkey chow analyzed, the level of hexachlorobenzene ranged from less than 1 ppb to 21.1 ppm. Traces of hexachlorobenzene, estimated to be less than 1 ppb, were also present in several batches of Wayne monkey chow.

Hexachlorobenzene (HCB), a fungicide and a chemical intermediate in industrial organic synthesis, has, in recent years, caused increasing concern as an environmental contaminant. It was first recognized as a hazardous chemical in the early sixties after an episode of massive human poisoning (porphyria cutanea tarda) in Turkey, resulted from the consumption of bread prepared from wheat contaminated with HCB (Schmid, 1960; Cam and Nigogosyan, 1963). Soon thereafter, HCB residues were found in the tissues of various species of wildlife, of domestic animals and humans, and in various food sources (Vos et al., 1968; Koeman et al., 1969; Acker and Schulte, 1970a,b; Tuinstra, 1971; Zeman et al., 1971; Brady and Siyali, 1972; Gilbertson and Reynolds, 1972; Goursand et al., 1972; Newton and Greene, 1972; Smith, 1972; Dejonckheere et al., 1974; Johnson et al., 1974). In a recent report (National Academy of Sciences, 1975) HCB was singled out as the only organic chemical contaminant present in the ocean at levels likely to cause serious problems.

During the course of a comparative metabolic study of hexachlorobenzene (HCB) in rhesus monkeys and rats (Yang and Pittman, 1975), fecal samples from untreated control monkeys were used for quantitative recovery of added HCB by gas chromatography. Significant quantities of HCB were detected consistently in the control monkey feces. Since every piece of glassware, other apparatus, and various reagents had been examined routinely by gas chromatography before each experiment, the source of HCB found was definitely the fecal material, from which the diet was incriminated.

This paper reports the evidence of HCB contamination in Purina monkey chow. The methods of sampling, extraction, clean-up, chromatographic, and mass spectrometric analysis of HCB from monkey feces and monkey chows are described.

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## EXPERIMENTAL SECTION

The various batches of Purina monkey chow (micromixed) used in this study were purchased from Hoosac Valley Feed and Grain Co., North Adams, Mass. On each bag (25 lb/bag) of the Monkey chow, there was a white information tag bearing the number 5038. The sampling and analysis of the Purina monkey chow were conducted in two different periods of time in the following manner.

(1) Over an 8-month period between October 1974 and May 1975, random sampling of 19 batches (200 g/batch) of monkey chow was carried out from freshly opened bags in our animal facility. Since the rate of turnover of monkey chow in our animal facility was approximately 3 bags/day, this sampling was therefore made from an estimated quantity of 18000 lb of monkey chow.

(2) In June 1975, five bags of Purina monkey chow were purchased directly from the same distributor. Upon consultation with the Ralston Purina Company, the following identification numbers were noted on the tape closure on the bottom of the bags: 0122751BT on three of the bags, 1009742 and 0102752 on the remaining two bags, respectively. The first seven digits denote, in sequence, the month, day, year, and the shift of the production. Thus, the number of 1009742 indicated that the product was manufactured on October 9, 1974 on the second shift. Two to three analyses were made on the monkey chow from each of these five bags.

Four batches of Wayne monkey diet were also analyzed during this study. They were purchased from R & E Feed Co., Troy, N.Y., and the bags carried an information tag bearing the number 8663-00.

Monkey feces were collected and pooled from various stock monkeys (*Macaca mulatta*) or from control monkeys on other experiments. These monkeys had no prior history of exposure to HCB and were not known to be in contact with pesticide in their well-ventilated holding rooms. At

least four lots of feces were analyzed.

HCB, being volatile, chemically stable, and not easily dissolved in common organic solvents, presents a very serious problem of contamination in the course of its extraction, clean-up, and gas chromatographic analysis. In this laboratory, the following precautionary measures were strictly enforced in routine HCB analysis. All glassware was first washed in detergent and rinsed thoroughly with tap water. It was then soaked in sulfuric acid–dichromate cleaning solution overnight. After rinsing thoroughly with tap water, the glassware was rinsed, in sequence, with distilled water, methanol, acetone, and hexane. Since the use of Nalgene or other plastic ware was totally avoided, the above solvents were stored in glass bottles and the rinsing was conducted with the aid of glass disposable pipets. Subsequently, every piece of glassware was rinsed again with a small portion (5–10 ml) of hexane, after which the hexane was analyzed on a gas chromatograph (GC) at the lowest attenuation employed in the routine quantitative analysis. Individual pieces of glassware were rejected if the presence of hexachlorobenzene was confirmed by GC. It was also found necessary to ensure freedom from contamination with HCB, as judged by GC, in the case of other apparatus, reagents, and solvents before use. When such precautions were taken, satisfactory gas chromatographic analyses were obtained.

Since certain pesticides are known to persist on the skin of pest control operators for long periods of time after exposure (Kazen et al., 1974) further stringent measures were adhered to in order to avoid any contact of dietary and fecal samples with hands, laboratory coats, and any other items which had not been subjected to examination by gas chromatography. The possible contamination of the laboratory environment by HCB through its volatility during storage was avoided by storing the chemical in tightly covered containers which were placed in sealed plastic bags.

Fifteen to eighteen pieces of monkey chow were finely ground in a Waring blender at low speed. Two hundred grams of the resulting powdered chow was transferred to a pre-extracted cellulose thimble (60 × 180 mm) and extracted with approximately 600 ml of benzene for 5 h in a Soxhlet apparatus. The resulting benzene extract was concentrated, cleaned-up on a Florisil column, and analyzed on a GC according to the method of Yang et al. (1975). For further analysis on the gas chromatograph–mass spectrometer (GC–MS), the pooled eluate from the Florisil column was again concentrated and cleaned up on an aluminum oxide column (40 × 2 cm i.d.) which was packed with partially deactivated (5.5% $H_2O$) neutral alumina as described by Holden and Marsden (1969). The first 100 ml of hexane eluate contained HCB and this fraction was concentrated and used for analysis with GC–MS.

Since Wayne monkey chow was much more oily than the Purina monkey chow, it was necessary to subject the eluate from the Florisil column to further purification on an aluminum oxide column before GC analysis.

Each lot of monkey feces (150–200 g) was homogenized in 4 × 200 ml portions of benzene. The contents were transferred to a pre-extracted cellulose thimble (60 × 180 mm) and then extracted for 10 h in a Soxhlet apparatus. The resulting benzene extract was treated in a manner similar to that described above. GC–MS analyses were conducted on the concentrated eluate from the Florisil column without further purification.

A Finnigan 3000 D system was used for the GC–MS analysis of various samples. The operating conditions were as follows: column, 6 ft × 2 mm i.d., 3% OV-1 on Chromosorb W HP 80–100 (CRS); carrier gas, 20 ml/min helium; column temperature, 190 °C; electron-impact ionization, 70 eV. The samples for GC–MS analysis were prepared from pooled extracts of approximately 1 kg of monkey chow and 400 g of control feces, respectively.

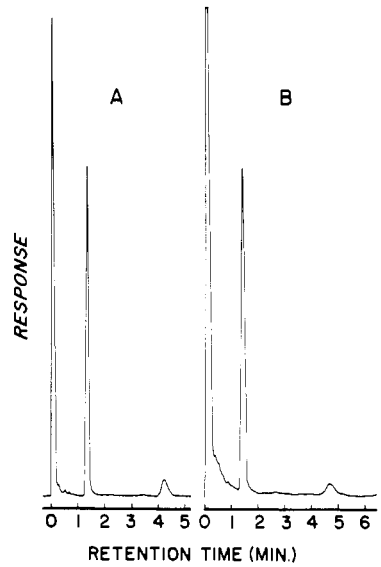


**Figure 1.** Gas chromatographic analysis of monkey fecal extract (A) and Purina monkey chow extract (B): column (6 ft coil, 2 mm i.d.), 1.5% OV-17 and 1.95% QF-1 on Supelcort, 100–120 mesh; detector, $^{63}Ni$; operating conditions, column, detector temperatures, 180 and 300 °C, respectively; carrier gas, 5% methane in argon, 40 ml/min.

## RESULTS AND DISCUSSION

Figure 1 represents the results of typical gas chromatographic analyses of extracts from monkey feces and Purina monkey chow. In either case, the major peak has a retention time identical with that of HCB. In the case of the monkey fecal samples, three lots were analyzed quantitatively while the monkeys were on Purina monkey chow and the HCB contents were 0.27, 0.54, and 0.7 ppm, respectively. One batch of monkey feces was analyzed 1.5 months after the monkeys were transferred to a diet of Wayne monkey chow, when the HCB content was found to be 0.11 ppm. Quantitative analysis of 30 batches of Purina monkey chow yielded HCB contents ranging from less than 1 ppb to 21.1 ppm. Of the 19 batches sampled between October 1974 and May 1975, the HCB levels were between 1.2 and 6.0 ppb for seven batches, 11.0 and 24.0 ppb for six batches, and 0.063 and 0.220 ppm for the remaining six batches. In the case of the five bags of monkey chow purchased in June 1975, one of them (1009742) contained the highest level of HCB of all samples analyzed so far. Three different analyses of samples taken from this bag revealed the presence of HCB at 4.4, 12.5, and 21.1 ppm, respectively. The monkey chow in the remaining four bags contained very low levels of HCB. Except for one sample which contained 3.3 ppb, all the other seven analyses (duplicate for each bag) showed traces of HCB (<1 ppb). In the four batches of Wayne monkey chow analyzed, the HCB levels were too low to quantify, but were estimated to be less than 1 ppb.

The results of GC–MS analyses of HCB standard and of the contaminants in the extracts of monkey feces and Purina monkey chow are shown in Figure 2. The spectra of the contaminants (Figure 2B,C) bear a close resemblance to that of HCB (Figure 2A) and show the mass fragmentation typical of HCB. The resolution of the portions

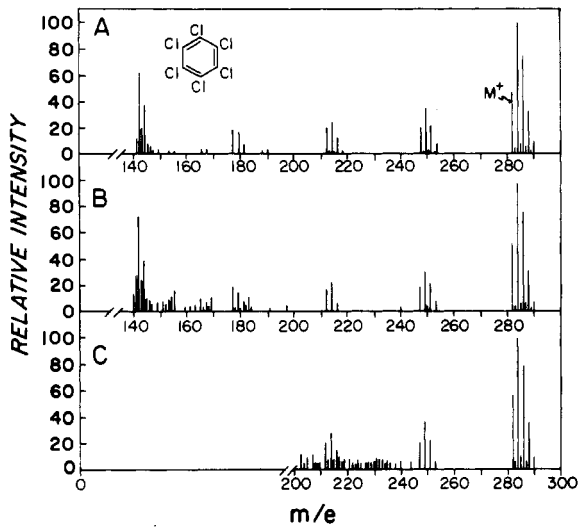


**Figure 2.** Mass spectra of HCB standard (A), control monkey fecal extract (B), and Purina monkey chow extract (C).

of mass spectra at lower $m/e$ values was poor, possibly due to the interfering substances from the GC column.

The findings presented here establish the presence of HCB contamination in laboratory monkey chows. Since the level of contamination was not consistent and all the contaminated samples appeared to have been produced prior to January 1975, it was suspected that this contamination might arise from certain batches of HCB-contaminated animal fat used in the preparation of these monkey chows. In this regard, it was interesting to note that the monkey chow which contained the highest level of HCB did appear more brown and greasy. However, in view of the fact that contamination with certain organic chemicals seems to be unavoidable, even under the most stringent precautionary procedures employed for Space programs (Gross and Colony, 1973), it is possible that other sources of HCB contamination may have been present during packaging, shipping, and storage of these animal chows.

Recently, Barsotti and Allen (1975) demonstrated that dietary levels of PCB's lower than or equal to the "safe" level set by the Food and Drug Administration for food products for human consumption affected pregnancy in rhesus monkeys. Although the level of HCB contamination of monkey chows in our study was not high in most instances, the consequences to experimental animals from prolonged exposure to this persistent pesticide may influence the course of long-term toxicological studies. It is advisable for investigators conducting chronic studies to monitor pesticide residue levels in each batch of animal diet.

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