A Study of Inhalation of Pentachlorophenol by Rats
IV. Distribution and Excretion of Inhaled Pentachloropenol

by HEINZ J. HOBEN, STEPHANIE A. CHING, and LOUIS J. CASARETT*
Department of Pharmacology
University of Hawaii
3675 Kilauea Avenue
Honolulu, Hawaii 96816

INTRODUCTION

The widely used pesticide, pentachlorophenol (PCP) and its salts, has become a long term contaminant of the environment presenting a potential hazard to health.

PCP may enter the body by way of absorption through the skin, ingestion of inhalation. The dermal and gastric toxicity of this compound has been studied extensively (DEICHMANN, et al. 1942), (WALTERS, 1952), (STOHLMAN, 1951). However, the potential toxicity by inhalation exposures to PCP has not been as readily investigated (CASARETT, et al. 1969).

To study the effect of inhaled PCP exposure, experiments with small laboratory animals were carried out. In single exposure experiments, the distribution and excretion were investigated. The possibility of an accumulation of this compound was studied in experiments in which the animals were exposed to a single dose from one to five times. Experiments of five repeated exposures furnished information of possible changes in accumulation, distribution and excretion of PCP under short term chronic exposure conditions.

MATERIALS AND METHODS

The materials and methods used for the PCP determination are described in part I of this study (HOBEN, et al. 1975 a). The inhalation exposure experiments were conducted as outlined in part II of this series (HOBEN, et al. 1975 b).** All animals used in the experiments were 2-3 months old male Sprague Dawley rats obtained from Martin Farm, East Palo Alto, California.

---

*deceased

**Not more than 12 animals could be exposed simultaneously since the exposure chamber has only 12 exposure sites.

Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 4 © 1976 by Springer-Verlag New York Inc.

EXPERIMENTAL

Single Exposure

Four groups of 11 (eleven) rats each were exposed to the aerosol of sodium pentachlorophenate. The animals weighed approximately 220 grams and the dose calculated from the exposure was 1.25 mg PCP/animal (5.7 mg/kg). After the 20 minute exposure, 3-4 animals were sacrificed at 0, 6, 12, 24, 48 and 72 hours after the exposure. The 24 hour sacrifice was included in each of the four exposures in an effort to check the reproducibility of dose in addition to the method of air sampling. The animals sacrified at 24, 48 and 72 hours were kept in individual metabolism cages for the collection of urine.

Multiple Exposure I

The multiple inhalation exposure I experiment was conducted on three groups of rats. Each group consisted of 12 animals with a mean body weight of 220 grams. The exposure time was 20 minutes daily. The calculated dose from each exposure was 1.30 mg/animal (5.9 mg/kg). Group I received 2 exposures, group II 3 exposures and group III 4 exposures. Six animals of each experiment were sacrificed immediately after the exposure (0 hours) and six 24 hours later. The animals sacrificed after 24 hours were kept in metabolism cages where their urine was collected.

Multiple Exposure II

Two groups of rats, consisting of 10 animals each, were exposed for 20 minutes daily for five days and sacrificed in groups of 3-4 animals at 0, 12, 24, 48 and 72 hours. As in the previous experiment, one group from each exposure was sacrificed at 24 hours. The calculated dose was 1.04 mg/kg/day.

RESULTS AND DISCUSSION

The aerosol mixture was sampled continuously for each exposure at a flow rate of 0.5 liter per minute. This resulted in a representative sample of 10 liters from which the chamber air concentration could be calculated. From the PCP content of this sample, the dose received per animal was determined using the assumption that each rat inhaled 80 ml of air per minute (1.6 l of air per exposure). (GUYTON, 1947).

Two tissue types and two body fluids were analyzed; the lung as the site of entry, the liver as the potential organ for metabolism, the blood as the medium of transport, and the urine containing the

Single Exposure

Including only the four sites analyzed, the data from Fig. I indicates that 70-75% of the inhaled dose can be accounted for as pentachlorophenol after 24 hours. In intraperitoneal injection experiments, the greatest recovery after any time was 30-40% (unpublished data of this laboratory). The lower overall recovery of unchanged pentachlorophenol in the injection series is probably due to its direct passage through the liver and subsequent biotransformation prior to distribution. These data suggest the significantly different fate of this compound depending on the type of exposure or "route of administration" and the resulting difference in toxicities (HOBEN, et al. 1975 c). Evidence to support the detoxification by the liver was obtained by identifying the metabolite by mass spectrometry, tetrachlorohydroquinone, in the liver and urine at the 24 hour sacrifice of the injected animals. This metabolite was also found in the mouse. (SVEN, 1971) In the inhalation exposures only trace amounts of this metabolite were present, whereas in the injection experiments there appeared to be almost equal amounts of pentachlorophenol and tetrachlorohydroquinone.* Consequently, it seems that less metabolism takes place when the lung is the route of entry, probably as a result of differences in distribution. These data also suggest the rapidity by which this compound is removed from the exposure site. The highest lung level of 1.8% was observed immediately after exposure. From Fig. I the half-life for pentachlorophenol can be estimated to be about 24 hours for a respiratory exposure and it does appear to follow first order kinetics. These data also indicate the usefulness of urine as an indicator of the level of exposure for rats after a single acute inhalation exposure. It is interesting to note the paralleling of the values for liver and plasma. The clearance rates of the tissues and fluids presented are essentially the same indicating no apparent storage or preferential binding at these sites.

Multiple Exposure I

The values obtained from the 0 and 24 hour groups of the single exposure experiment and the multiple exposure II experiment were included in the discussion of this study to make possible a comparison of the fate of inhaled PCP from one single dose to five consecutive doses.

---

*The quantitative of tetrachlorohydroquinone has not been successful due to its potential for oxidation and the inability of the analytical method used here to detect the quinone form.

excreted products. Figure I shows the distribution of PCP for these tissues and fluids at 0, 6, 12, 24, 48 and 72 hours after the exposure. Other tissue types, heart, spleen, kidney and fat had been analyzed in preliminary intraperitoneal injection and inhalation studies. Each of these body tissues contained less than ½% of the dose except for the kidney which contained not more than 2% of the dose; consequently, their analyses were not included in this study.

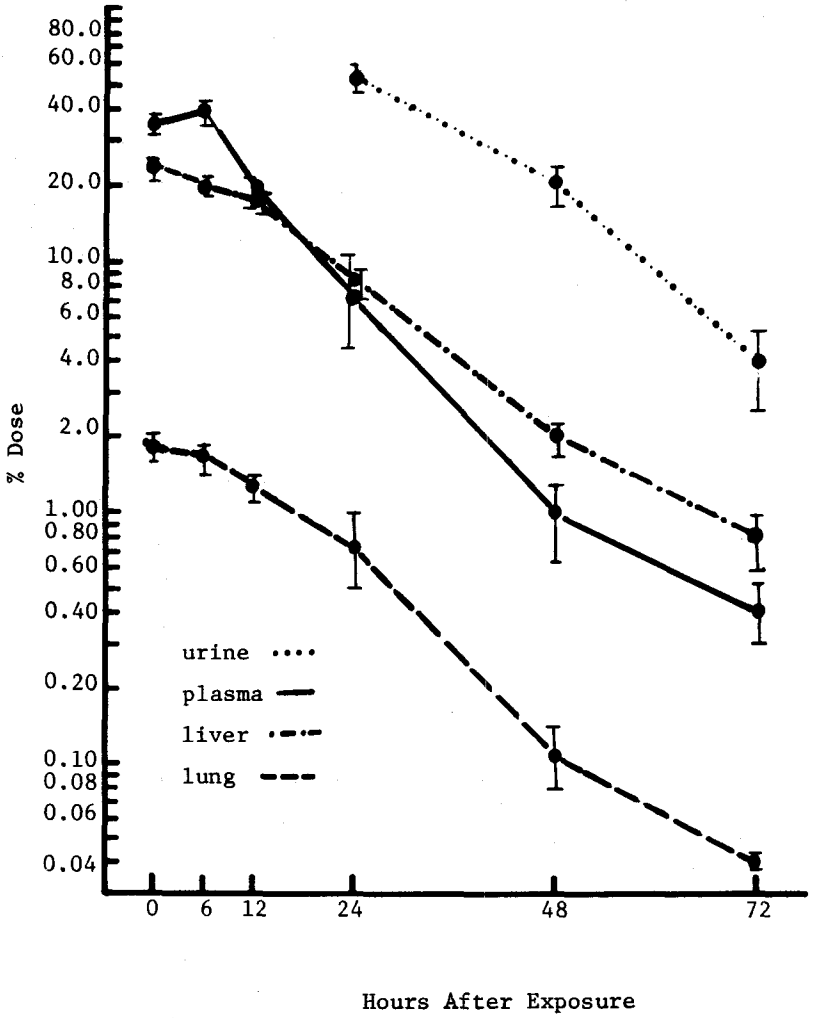

Fig. I. Distribution of inhaled PCP after one single acute dose

Since the half-life from the single dose experiment was approximately 24 hours, daily exposures to pentachlorophenol would be expected to result in an increasing body burden of this material. The data on Figures II, III, and IV were calculated considering only a single days dose ignoring the potential accumulation. Instead of continuing to increase as one might expect the "0" hour values (Fig. II and III) initially appeared to increase, returned to the single dose value after the third exposure with a downward trend following the remaining exposures. These data suggest the existence of mechanisms of enhanced excretion, accelerated biotransformation, and/or storage in non-analyzed sites. In Fig. IV the 24 hour urine results suggest increased urinary excretion, however, the wide standard error lessens the significance of this potential mechanism. Accelerated biotransformation can be indirectly supported from these data. The apparent increase in urinary output (a maximum increase of 20% after the fifth exposure) does not account for the total amount of pentachlorophenol that theoretically should have accumulated as a result of the daily exposures. Consequently, an increase in metabolism would account for this, possibly similar to the enzyme enhancing action of other chlorinated hydrocarbon pesticides. (MORELLO, 1965) (SANCHEZ, 1967) (HART, 1963) (BOSTROM, 1972). Further study is needed for quantitative support of this hypothesis.

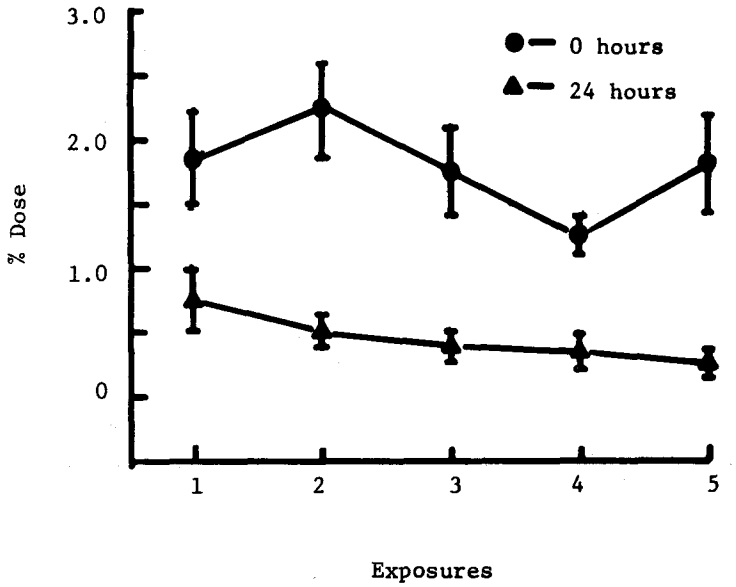

Fig. II. Percent dose of PCP in lung at 0 hours and 24 hours after repeated inhalation exposures.

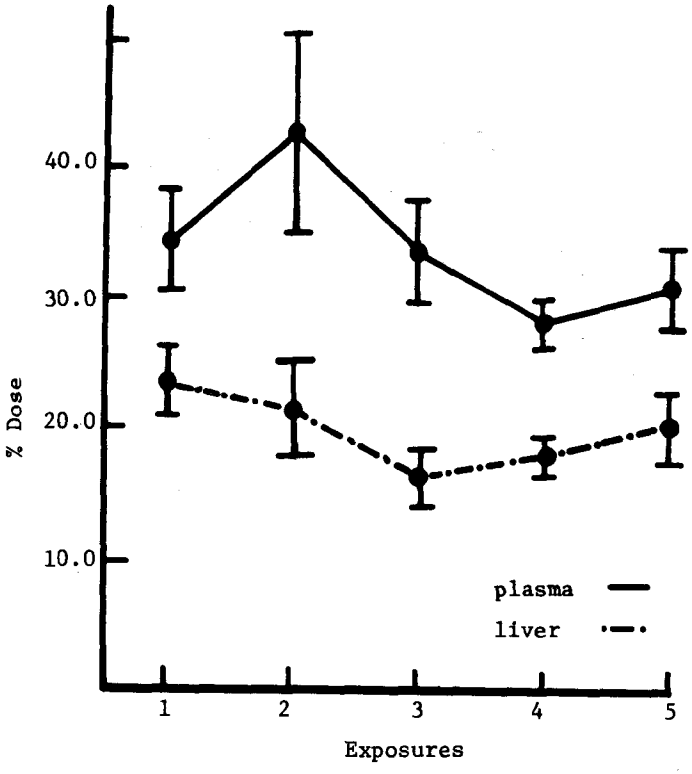

Fig. III. Percent dose of PCP in plasma and liver at 0 hours after repeated inhalation exposures.

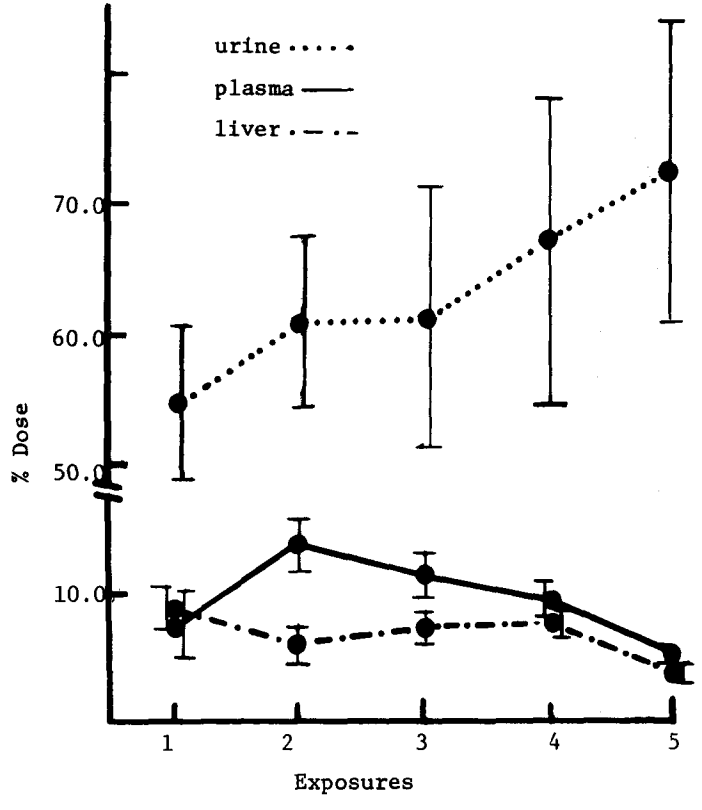

Fig. IV. Percent dose of PCP in plasma and liver and its excretion in urine 24 hours after repeated inhalation exposures.

The 24 hour values, Fig. II and IV, also demonstrate the apparent lack of accumulation of this compound in the rat. These data also decrease slightly with repeated exposures supporting the idea of an induced mechanism to accelerate the handling of this material.

Storage in an unidentified depot, possibly fat, is a possible explanation for the lack of accumulation in the tissues and fluids examined. However, a more rapid initial distribution would then be necessary to account for the lack of increase at the 0 time period, a position which would be difficult to substantiate. An extended elimination period or increase in elimination rate would be expected if storage was the explanation, neither of which is supported by the results of the multiple exposure II experiment.

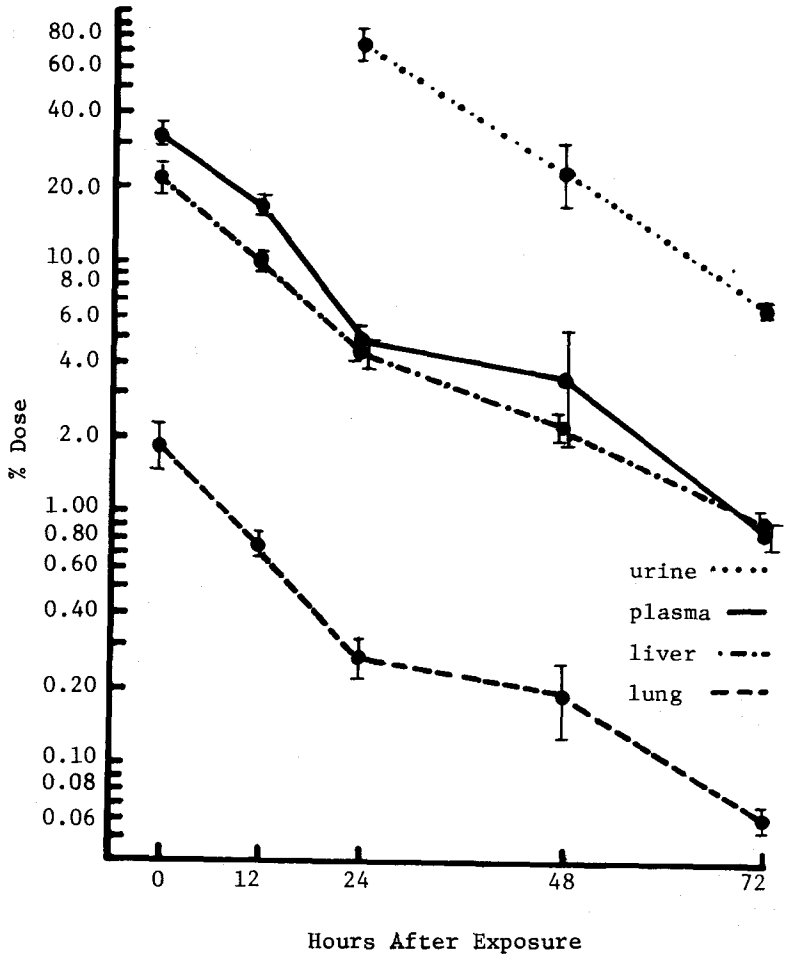

Fig. V. Distribution and elimination of inhaled PCP after 5 daily exposures.

Multiple Exposure II

Again the data was determined by calculating the percent dose values as if the fifth or last exposure was the only dose received by the animals. A close comparison of the percent dose recovered after the fifth exposure (Fig. V) to those after the single exposure (Fig. 1) illustrates almost identical elimination rates and time periods. These results do not support the potential for storage in an unidentified compartment.

SUMMARY

It appears that in the rat repeated respiratory exposures to PCP do not result in an increase in the body burden of this compound as would be suspected from the 24 hour half-life determined from a single inhalation exposure. These results suggest some mechanism induced by prior exposure to PCP that increases the ability of the animals to remove this compound from its body. Increased excretion may be a factor in this activity, however, it cannot account for the total effect. Storage appears unlikely, since the elimination rate and time period remain unchanged after five doses as compared to after one. Increased metabolism may be the explanation, although this mechanism can only be inferred from these data. Quantitative metabolic results will be necessary to support this hypothesis.

ACKNOWLEDGEMENTS

This investigation was supported by NIH research grant number ES 00459 from the National Institute of Environmental Health Sciences.

The authors thank Dr. Ted Norton for his valuable suggestions in reviewing this paper and Miss Robin Young and Mr. Gary Mols for technical assistance. Mr. Albert Saito's help in preparing the graphs is also gratefully acknowledged.

REFERENCES

BOSTOM, S.L. Comp. Biochem. Physiol. 41(2), p. 359, 1972.

CASARETT, L.G., BEVENUE, A., W.L. YOUGER, JR., WHALEN, S. Amer. Ind. Hyg. Assoc. J. 30(4), p. 360-366.

GUYTON, A.C. Amer. J. Physiol. 150:70, 1947.

HART, L.G., SHULTICE, R.W., FOUTS, JR. Tox. Applied Pharm. J. 3, p. 71, 1963.

HOBEN, H., S.A. CHING, R.A. YOUNG and L.J. CASARETT Bull. Environ. Contam. Toxicol. Part I (1975a), in press.

HOBEN, H., S.A. CHING, and L.J. CASARETT Bull. Environ. Contam. Toxicol. Part II (1975b), in press.

HOBEN, H., S.A. CHING, and L.J. CASARETT Bull. Environ. Contam. Toxicol. Part III (1975c), in press.

MORELLO, A. Can. J. Biochem. 43, p. 1289, 1965.

SANCHEZ, E. Can. J. Biochem. 45, p. 1809, 1967.

STOHLMAN, E.F. Pub. Health Repts., U.S. Public Health Service, 66, 1303, 1951.

SVEN, YLLNER Acta Pharm. Toxicol., 29(5-6), p. 513, 1971.

WALTERS, C.S. Proc. Am. Wood-Preservers' Assoc. 48, 322,1952.