# The Effects of Lead Ingestion on the Body Burden of DDT, Liver Vitamin A and Microsomal Enzyme Activity in the Rat

by W. E. J. PHILLIPS, D. C. VILLENEUVE, and G. C. BECKING

*Research Laboratories*
*Food and Drug Directorate*
*Department of National Health and Welfare*
*Ottawa, Canada*

Lead is a non-essential metal for man. It is, however, assimilated from food and other sources in the environment. The toxicology of inorganic lead was reviewed by Roush and Kehoe (1). Patterson (2) and Schroeder and Tipton (3) have reviewed the subject of human body burdens of lead and concluded that the exposure of people in the United States to lead from all sources was apparently large enough to cause accumulation with age. Lead was therefore selected as a candidate contaminant for study in a continuing program on the effects of environmental contaminants on pesticide metabolism. This study reports not only on the effects of lead ingestion by the rat on metabolism and storage of DDT, but also on pentobarbital sleeping times, in vitro liver carboxylesterase activity and the utilization of dietary carotenoids and vitamin A.

## Materials and Methods

Seventy male Wistar rats (100-125 g) were randomized into 7 groups of 10 rats. Four groups (4, 5, 6 and 7) received an oral dose of p,p'-DDT in corn oil (40 mg/kg body weight) while the other 3 groups (1, 2 and 3) received only corn oil. All seven groups were maintained on standard fox cubes for five days. On the fifth day, group 7 was killed and abdominal fat removed for DDT analyses. These values show the initial concentration of DDT in the fat prior to the lead feeding. At this time, rats in the remaining groups were fed diets (ground fox cubes) containing 0, 100 or 200 ppm lead (as lead acetate) (Table 1). The vitamin A activity in the ground cubes was derived from both carotene (4000 ug $\beta$-carotene/kg) and preformed vitamin A (2000 ug/kg).

After 36 days on test pentobarbital sleeping times (4) were determined on 5 animals from each group. The other five animals were killed, and livers and abdominal fat samples removed, weighed and frozen for subsequent analyses. Liver carboxylesterase activity was determined on liver homogenates (20% w/v) as follows: 4 ml of the substrate (0.02 M o-nitrophenyl butyrate in 0.1 M phosphophosphate buffer, pH 6.3) was



Bulletin of Environmental Contamination & Toxicology,
Vol. 6, No. 6, 1971, published by Springer-Verlag New York Inc.

placed in a cuvette. A suitable volume of homogenate was added to the substrate solution and the released o-nitrophenol measured spectrophotometrically at 372 mu and 25°C. Protein was determined on the homogenate (5) and the results expressed as u moles substrate utilized per hour per mg protein.

Liver vitamin A was determined (6) and expressed as ug vitamin A per g liver and ug vitamin A per whole liver. DDT analysis was carried out on the fat samples according to the following method: Two hundred mg of the fat was blended with 100 ml hexane and 50 g anhydrous sodium sulfate for 10 minutes. The extract was filtered, concentrated and the volume made up to 10 ml with hexane. Fat was precipitated using concentrated $H_2SO_4$-fuming $H_2SO_4$ (1:1) After mixing, the tubes were centrifuged and the hexane layer transferred to another test tube. Four grams of sodium sulfate:sodium carbonate (9:1) was added to the hexane extract and an aliquot was removed for GLC analysis for DDE, DDD and DDT (7). Results are expressed as ppm DDE, DDD, DDT.

Five animals, which had previously undergone pentobarbital sleeping time determinations after 36 days on test, were allowed to continue on their respective diets for a total of 90 days. Sleeping times were again determined after 85 days, and on the 90th day all animals were killed, and the liver and fat removed for analyses. DDT and liver vitamin A were measured as previously described.

## Results and Discussion

There was no effect of treatment on body weight gain or liver weight after 36 days or 90 days. The effects of lead ingestion and DDT treatment on pentobarbital sleeping times are shown in Table I.

After 36 days on diet, the animals dosed with DDT and receiving no lead in their diets had decreased sleeping times as compared to the controls. Rats predosed with DDT and ingesting diets containing 100 and 200 ppm lead also had significantly decreased sleeping times. Lead had no effect on the ability of DDT to reduce sleeping time. After 85 days feeding, there was no difference in the sleeping times of the controls and the DDT pre-treated group of rats. Lead did not significantly alter the sleeping times of the DDT pre-treated rats. Sleeping times were unaffected by dietary lead when fed for 36 or 90 days.

There was no effect of treatment on either carboxylesterase activity or the protein content of

liver homogenates after 36 days. The *in vitro* carboxylesterase activity in liver homogenates from control animals at 36 days was 25.8±0.7 u moles o-nitrophenyl butyrate/hr/mg protein. Since treatment evoked no significant effect on the protein content of the liver or carboxylesterase activity a complete table of values is not shown.

TABLE 1
The effects of lead ingestion and DDT treatment on pentobarbital sleeping times[1] in the rat

| Treatment | Sleeping Time (minutes) + S.E.M. Days on Test 36 | 85 |
|---|---|---|
| Controls | 70.3 ±4.5 | 69.4±10.0 |
| 100 ppm lead | 64.4 ±6.2 | 83.8± 4.2 |
| 200 ppm lead | 64.7 ±3.4 | 88.0± 5.5 |
| DDT pre-dosed | 52.5*±2.3 | 72.6± 7.5 |
| DDT pre-dosed + 100 ppm lead | 52.3*±4.8 | 61.8± 4.6 |
| DDT pre-dosed + 200 ppm lead | 52.8*±2.0 | 70.0± 4.3 |

[1]Animals dosed with sodium pentobarbital at 40 mg/kg body weight.
*Significantly different from controls (P = 0.05).

TABLE 2
The effects of lead ingestion on the levels of p,p'-DDT and metabolites in adipose tissue

| Group Number | 7 | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|
| Treatment | | | | | | | |
| DDT[1] | + | + | | + | | + | |
| Lead (ppm in diet) | 0 | 0 | | 100 | | 200 | |
| Days on diet | 0 | 36 | 90 | 36 | 90 | 36 | 90 |
| Adipose tissue (ppm) | | | | | | | |
| p,p'-DDT | 52.1 | 9.7 | 1.3 | 10.1 | 2.2 | 7.6 | 1.7 |
| p,p'-DDD | 4.3 | 0.83 | 0.6 | 0.72 | 0.5 | 1.3 | 0.7 |
| p,p'-DDE | 3.4 | 1.4 | 1.5 | 1.2 | 1.4 | 1.9 | 1.3 |

[1]Single oral dose of p,p'-DDT in corn oil (40 mg/kg body weight) 5 days prior to feeding of test diets.

The effects of lead on the p,p'-DDT, DDD and DDE levels in adipose tissue are shown in Table 2. There was no statistically significant effect of dietary lead on the p,p'-DDT, p,p'-DDD or p,p'-DDE levels of adipose tissue after 36 days or 90 days of treatment. This indicates that the ingestion of lead at levels as high as 200 ppm does not affect the depletion of DDT and its metabolies or the ratio of metabolites to the parent compound in the adipose tissue of the rat.

The data from indirect measurements of microsomal enzyme activity and the rate of depletion and metabolism of DDT are compatible. Induction of hepatic mixed function oxidases by DDT (8, 9) or barbiturates (10) has been shown to decrease storage in the adipose tissue of organochlorine pesticides. Thus if lead modified microsomal enzyme activity (either induction or inhibition) a change in the rate of depletion of DDT and metabolites would be expected. That lead under "normal" urban conditions can inhibit an enzyme system in man was recently demonstrated by Hernberg and Nikkanen (11). A negative correlation was found between the concentration of lead in blood and the activity of erythrocyte $\delta$-aminolaevulinic acid dehydratase. This demonstrated that levels of environmental contamination with lead can produce a measurable biochemical alteration in man. In the present study extremely high levels of lead were fed yet no biochemical lesion was induced to influence microsomal enzyme activity or DDT depletion.

A further aspect of this study was to assess the effects of dietary lead on the liver storage of vitamin A in rats consuming a normal stock ration containing both $\beta$-carotene and preformed vitamin A.

The concentration of liver vitamin A and the total amount of vitamin A stored per liver were not different between groups (Table 3) following 36 or 90 days of ingestion of diets containing 0 to 200 ppm lead with or withcut a pre-treatment of a single dose of DDT.

This negative finding is of interest in relation to a survey of vitamin A and carotene reserves of Canadians at five major cities (12). There were striking regional differences in the number of subjects having non-detectable or low vitamin A stores. These differences may be related to dietary intake or unknown environmental factors. The data clearly shows that the feeding of high levels of lead for periods up to 90 days did not modify liver vitamin A levels. Although there are many environmental contaminants that may be

contributing factors to the low vitamin A reserves of Canadians, it would appear that lead is not specifically implicated.

The FAO/WHO (13) consider that for man the maximum acceptable load of lead from food can tentatively be placed at 0.005 mg/kg body-weight per day. They state that the average daily intakes of lead from normal food and beverages probably lie between 0.0033 and 0.005 mg/kg body-weight with approximately a further 0.0015 mg/kg body-weight per day from the atmosphere in an urban environment. Since in the present experiment rats were stressed with lead intakes many times the

TABLE 3
The effects of lead ingestion on the liver vitamin A status of the rat.

| Treatment | Liver vitamin A Days on Test 36 | | 90 | |
|---|---|---|---|---|
| Controls | 353[1] | (4230[2]) | 636 | (9222) |
| 100 ppm lead | 348 | (4703) | 668 | (9827) |
| 200 ppm lead | 343 | (4107) | 695 | (10580) |
| DDT pre-dosed | 354 | (4200) | 670 | (10072) |
| DDT pre-dosed + 100 ppm lead | 338 | (3965) | 694 | (9721) |
| DDT pre-dosed + 200 ppm lead | 342 | (4282) | 678 | (11216) |

[1]ug vitamin A/g liver.
[2]ug vitamin A/whole liver.

maximum acceptable load for man it is suggested that the present levels of lead in the food supply do not cause alteration in the metabolism of DDT or influence the liver storage of vitamin A.

## Summary

Lead ingestion at 100 and 200 ppm for 36 and 90 days had no effect on the disappearance of DDT residues (and metabolites) from adipose tissue of rats. Lead alone, and lead fed to DDT pre-treated animals had no effect on body weight gain, liver weights, liver vitamin A, liver protein, *in vitro* liver carboxylesterase activity and pentobarbital sleeping times.

## Acknowledgements

The authors gratefully acknowledge the techinical assistance of Mr. G. Hatina and Mr. J. LaCroix.

## References

1 Roush G., Jr., and Kehoe, R.A. Ann. Rev. Pharmacol. 4, 247, 1964.

2 Patterson, C.C. Arch. Environ. Health 11, 344, 1965.

3 Schroeder, H.A., and Tipton, I.H. Arch. Environ. Health 17, 965, 1968.

4 Villeneuve, D.C., Phillips, W.E.J., and Syrotiuk, John. Bull. Environ. Contamin. Toxicol. 5, 125, 1970.

5 Gornall, A.G., Bardawill, C.J., and David, M.M. J. Biol. Chem. 177, 751, 1948.

6 Phillips, W.E.J. Can. J. Biochem. Physiol. 40, 491, 1962.

7 McCully, K.A., and McKinley, W.P. J.A.O.A.C. 47, 725, 1964.

8 Street, J.C. Science 146, 1580, 1964.

9 Street, J.C., Chadwick, R.W., Wong, M., Phillips, R.L. J. Agr. Food Chem. 14, 545, 1966.

10 Cueto, C., Jr., and Hayes, W.J., Jr. Toxicol. Appl. Pharmacol. 7, 481, 1965.

11 Hernberg, S., and Nikkanen, J. Lancet, pg. 63, 1970.

12 Hoppner, K., Phillips, W.E.J., Erdody, P., Murray, T.K., and Perrin, D.E. Canad. Med. Assoc. J. 101, 736, 1969.

13 FAO/WHO. Wld. Hlth. Org. Techn. Rep. Ser. no. 373, pg. 15, 1967.