Induction of Hepatic Microsomal Enzymes after Administration of a Combination of Heptachlor and Phenobarbital

by V. KRAMPL, M. VARGOVÁ, and M. VLADÁR
Institute of Hygiene and Occupational Medicine
Bratislava, Czechoslovakia

It is known that some organochlorine insecticides are able to induce synthesis of hepatic microsomal enzymes that catalyse the metabolism of drugs and other chemical agents. (HART & FOUTS 1963,1965: HART et al.1963)

This induction of the enzymatic activity of hepatic cells, which are responsible for the metabolism of various drugs, pesticides included, is recently put to the centre of study. The practical importance of such an induction is stressed by the fact that the interaction of various drugs with pesticides can alter the effect as well as the toxicity of these drugs(CONEY 1967: CUCINELL et al. 1965)

In our experiments we tried to establish the influence of a single dose of the pesticide heptachlor given simultaneously with another enzymatic inductor-phenobarbital in various ratios upon the enzymatic activity of hepatic microsomal enzymes.

Material and Methods

Male Wistar rats, 130-150 g maintained on Larsen diet were used in these experiments and were fasted overnight prior to killing.

Heptachlor was administered in pure vegetable oil by means of oral tube. Phenobarbital was injected intraperitoneally. The experimental animals received

Address for reprints: Dr. V.Krampl,Bratislava, Dukelská 20,Czechoslovakia

Bulletin of Environmental Contamination & Toxicology, Vol. 9, No. 3, © 1973 by Springer-Verlag New York Inc.

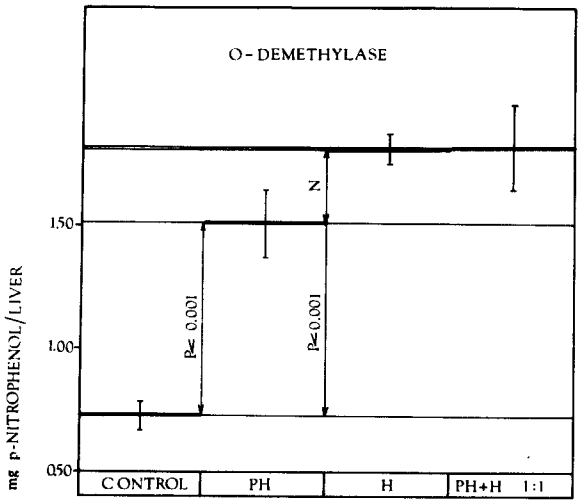

FIGURE 1. Induction of O-demethylase after a single dose of phenobarbital (PH), heptachlor (H), and of a combination of both phenobarbital and heptachlor (PH+H), ratio 1:1 (60 mg/kg phenobarbital + 80 mg/kg heptachlor). Mean values of the enzymatic activity and their standard deviations are given. N= the difference was not significant.

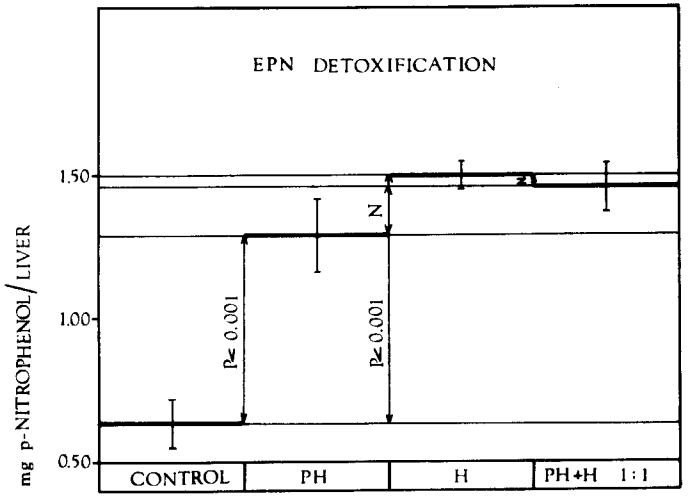

FIGURE 2. Induction of the EPN detoxification system after a single dose of phenobarbital (PH), heptachlor (H), and a combination of both phenobarbital and heptachlor (PH + H), ratio 1:1 . (60 mg/kg phenobarbital + 80 mg/kg heptachlor).

both 80 and 160 mg/kg of heptachlor, and 60 and 120 mg/kg of phenobarbital respectively. These doses are equal to 1/3 and 2/3 of the LD_{50} ones respectively (GRUBER et al.1944 : ROSIVAL et al. 1969)

In the experiment three different dose ratios were used. First ratio: 1:1 /1/3 LD_{50} of phenobarbital + 1/3 LD_{50} of heptachlor/; 2nd ratio: 1:2 /phenobarbital:heptachlor/; 3rd ratio: 2:1 /phenobarbital: heptachlor/. Control animals received only oil.

Both experimental and control animals were sacrificed by decapitation 24 hrs after administration. Hepatic microsomal enzyme assays were performed in whole homogenates of the livers of rats immediately after the animals were sacrificed.

Two reactions catalyzed exclusively by microsomal enzymes were included in this study. They were the oxidative detoxification of O-ethyl O-/4-nitrophenyl/ phenylphosphonothioate /EPN/, and the O-demethylation of p-nitroanisole. The activity of these enzymes was determined by the method of Kinoshita et al.(1966) and was expressed as mg of p-nitrophenol per liver per 60 minutes.

Results and Discussion

No potentiation of simultaneously given inductors /heptachlor and phenobarbital, ratio 1:1, i.e. 80 mg/kg of heptachlor and 60 mg/kg of phenobarbital/ was observed in changes of activity of the following microsomal enzymes. The results are shown in Figures 1,2.

When these inductors were administered in a ratio 1:2 /60 mg/kg of phenobarbital and 160 mg/kg of heptachlor/, statistically significant increase of the O-demethylase and of EPN detoxification level was found. The increase did not reach the value of the

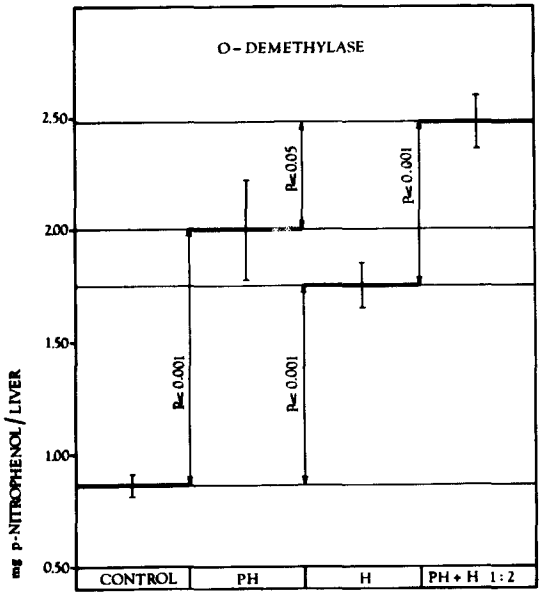

FIGURE 3. Induction of O-demethylase after a single dose of phenobarbital (PH), heptachlor (H), and of a combination of phenobarbital and heptachlor (PH + H), ration 1:2. (60 mg/kg phenobarbital + 160 mg/kg heptachlor)

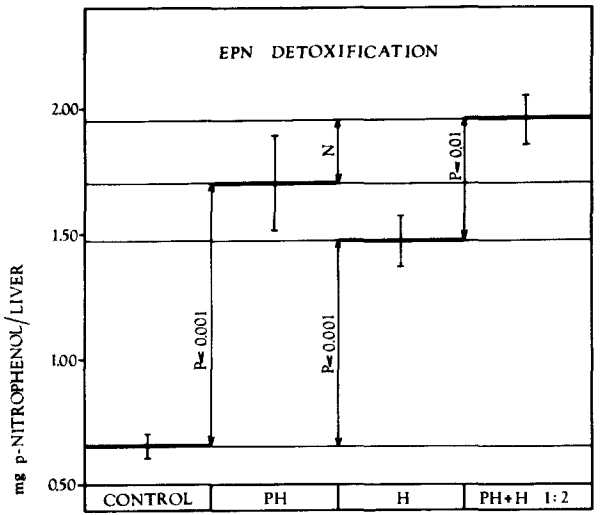

FIGURE 4. Induction of EPN detoxification system after a single dose of phenobarbital (PH), heptachlor (H), and a combination of phenobarbital and heptachlor (PH + H), ratio 1:2 . (60 mg/kg phenobarbital + 160 mg/kg heptachlor)

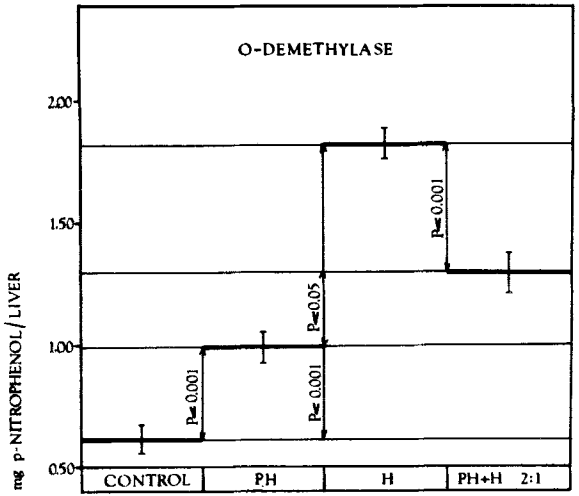

FIGURE 5. Induction of O-demethylase after a single dose of phenobarbital (PH), heptachlor (H), and a combination of phenobarbital and heptachlor (PH + H), ration 2:1 . (120 mg/kg phenobarbital + 80 mg/kg heptachlor).

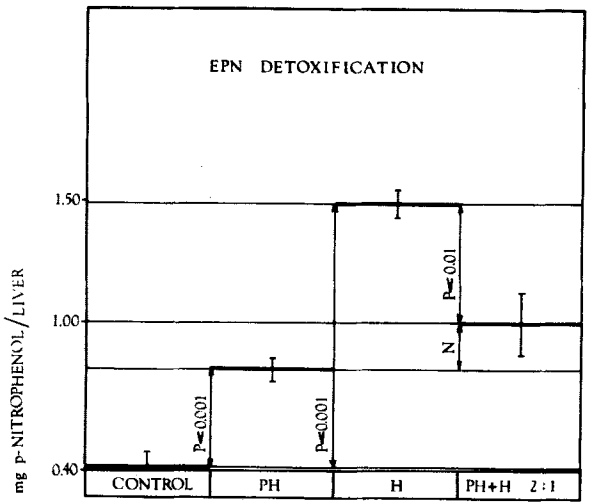

FIGURE 6. Induction of EPN detoxification system after a single dose of heptachlor (H), phenobarbital (PH), and a combination of phenobarbital and heptachlor (PH + H), ratio 2:1 . (120 mg/kg of phenobarbital + 80 mg/kg heptachlor) .

total of activity changes found when inductors were given separately. With respect to the action of inductors given separately /see above/ this finding seems to be reasonable. See Figures 3,4.

However a decreased stimulating effect of phenobarbital was observed the ratio being 2:1 /120 mg/kg of phenobarbital, and 80 mg/kg of heptachlor/. The enzymes levels were significantly lower than the corresponding ones on administering 80 mg of heptachlor, when both inductors are administered simultaneously /Figures 5,6/.

On the basis of these observations following conclusion could be drawn:Although a single dose /as mentioned/ represents only 1/3 of LD_{50},and can cause significant changes of activity of both enzymes /when compared to controls/, the double dose practically fails to increase the activity of enzymes. As to heptachlor, when given in double dose, only statistically nonsignificant changes of O-demethylase activity, while no changes of EPN detoxification can be found. When the phenobarbital dose was doubled even a diminished stimulation activity could be found.

One can expect that the stimulation of microsomal enzymes will be markedly greater when two inductors are given simultaneously than in conditions when they are given separately. However, our results showed that the stimulation of hepatic microsomal enzymes has been probably limited. The limiting factor seems to be the capacity of enzymes as well the dosis of the drug. Individual inductors or their combination takes part upon this limiting factor.

Acknowledgement

We wish to thank Miss E.Kubányiová for her technical cooperation in this work.

References

CONEY, A.M. et al. Clin.Pharmacol.Therap. 8,2/1967/

CUCINELL, S.A. et al. Clin.Pharmacol.Therap. 6,420 /1965/

GRUBER, C.M. et al. J.Pharmacol.Exptl.Therap. 61, 254/1944/

HART, L.G., FOUTS, J.R. Proc.Soc.Exptl.Biol.Med. 114,388/1963/

HART, L.G. et al. Toxicol.Appl.Pharmacol. 5,374 /1963/

HART, L.G., FOUTS, J.R. Arch.Exptl.Pathol.Pharmacol. 249,486/1965/

KINOSHITA, F.K. et al. Toxicol.Appl.Pharmacol. 9, 505/1966/

ROSIVAL, L. et al. Brat.Lek.Listy 51,170/1969/