Biochemical Pharmacology, Vol. 19, pp. 2605–2607. Pergamon Press. 1970. Printed in Great Britain

EFFECTS OF METHYLENEDIOXYPHENYL INSECTICIDAL SYNERGISTS *IN VITRO* ON HYDROXYLATIONS OF BIPHENYL BY MOUSE LIVER MICROSOMES*

S. S. EPSTEIN, R. G. CSILLAG, H. GUERIN and M. A. FRIEDMAN

Laboratories of Environmental Toxicology and Carcinogenesis,
Children's Cancer Research Foundation, Inc., and the Department of Pathology,
Harvard Medical School, Boston, Mass. 02115, U.S.A.

(*Received* 13 *December* 1969; *accepted* 23 *February* 1970)

Abstract—Piperonyl butoxide, piperonyl sulfoxide and *n*-propyl isome produce concomitant stimulation of *o*-hydroxylation and inhibition of *p*-hydroxylation of biphenyl by mouse liver microsomal preparations *in vitro*.

A WIDE range of chemically unrelated compounds, including natural and synthetic methylenedioxyphenyls (MDP),[1–5] hydrazines,[6] and SKF 525A[1, 2] induce transient inhibition of mammalian microsomal enzyme function *in vivo* and *in vitro*. Synthetic MDP derivatives, such as piperonyl butoxide (PB), are widely used as synergists with pyrethrin insecticides; putatively, synergists act by blocking insect microsomal enzyme function.[7] More recently, PB and related compounds have been shown *in vivo* to induce a paradoxical and transient stimulation of *o*-hydroxylation of biphenyl concomitantly with inhibition of *p*-hydroxylation in mouse liver microsomes.[5] We report here stimulation and inhibition *in vitro* of mouse liver microsomal *ortho*- and *para*-biphenyl hydroxylation, respectively, by MDP insecticide synergists.

EXPERIMENTAL

Adult male Swiss albino mice (ICR/Ha), maintained on Purina chow and water *ad lib.*, were sacrificed by cervical dislocation; the livers were removed and gall bladders discarded. Crude microsomal fractions were prepared from liver homogenates in 0·25 M sucrose containing 0·1 M phosphate buffer at pH 7·4. Dilutions of PB, piperonyl sulfoxide, and *n*-propyl isome (Fig. 1) were prepared in 0·6% (v/v) Tween 80 from 10 mM stock suspensions in 0·6% Tween to yield concentrations ranging from 0·1 to 1·0 mM in the incubation mixture, with a final Tween concentration of 0·06%. Preparations were agitated until satisfactory suspensions were obtained; *n*-propyl isome yielded relatively nonhomogeneous suspensions. All incubation mixtures, excluding the blanks, contained 6 μm of biphenyl substrate per 3 ml of reaction mixture. *Ortho*- and *para*-biphenyl hydroxylase activity was determined with an isocitric dehydrogenase system at pH 7·5;[8] both isomers were measured fluorimetrically in an Aminco–Bowman spectrophotofluorimeter at 345 and 415 mμ, with respective excitation at 275 and 295 mμ. Experiments were replicated on 4 occasions.

* This work was supported by NIH Grants C-6516 and FR-05526.

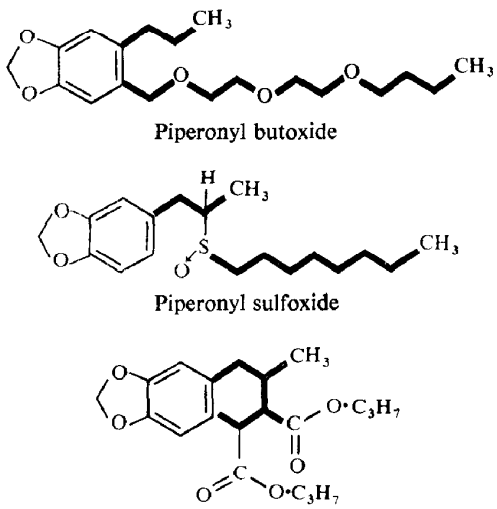

FIG. 1. Synthetic methylenedioxyphenyl insecticidal synergists.

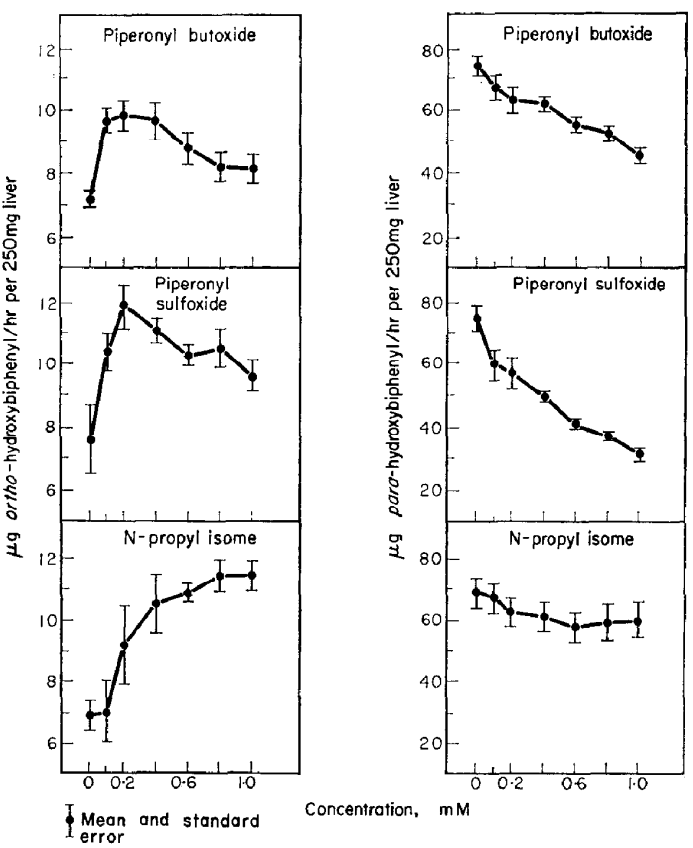

FIG. 2. Effects of methylenedioxyphenyl insecticidal synergists on hydroxylations of biphenyl *in vitro*.

RESULTS

In vitro, the 3 MDP synergists induce concomitant concentration-dependent stimulation of *o*-biphenyl hydroxylase activity, and concentration-dependent inhibition of *p*-biphenyl hydroxylase activity (Fig. 2). Dose–response plots of *o*-biphenyl hydroxylase activity peak between 0·1 and 0·4 mM; for *n*-propyl isome, a plateau is reached at a relatively higher concentration. Additionally, *n*-propyl isome inhibits *p*-hydroxylation to a lesser degree than PB and piperonyl sulfoxide.

DISCUSSION

There is good correspondence between these effects *in vitro*, both inhibition and stimulation, induced by the 3 MDP synergists and their previously reported effects *in vivo*.[5] The weak activities of *n*-propyl isome *in vivo*[5] and *in vitro* may reflect its relative insolubility. Stimulation, both *in vivo* and *in vitro*, of certain mammalian microsomal enzyme functions by MDP synergists is of particular interest since it contrasts with the commonly accepted view that such compounds are generalized and nonspecific microsomal enzyme inhibitors.

Since MDP synergists inhibit a broad spectrum of microsomal enzymes, NADPH and reduced cytochrome P-450 might accumulate, as these cofactors would otherwise be oxidized via microsomal mixed function oxidation. If reduced cytochrome P-450 is rate limiting to *o*-biphenyl hydroxylase, increases in its concentration will enhance the reaction rate; however, NADPH is not rate limiting since it is generated *in vitro* by isocitric dehydrogenase. These synergists might alternatively produce changes in the secondary structure of *p*-biphenyl hydroxylase, which alter the specificity of this enzyme, resulting in hydroxylation of biphenyl in the *ortho*-position.[5] Such alteration of secondary structure might be induced by interaction of MDP synergists with the active site of the enzyme or with an allosteric site on the enzyme or the enzyme–membrane complex. Conversion of P-450 to P-420 by detergents such as deoxycholate, Lubrol W, and Tweens has been established.[9] The detergent properties of PB[5, 10] may be involved in its effects on microsomal enzyme functions.

REFERENCES

1. M. W. Anders, *Biochem. Pharmac.* **17**, 2367 (1968).
2. K. Fujii, H. Jaffe and S. S. Epstein, *Toxic. appl. Pharmac.* **13**, 431 (1968).
3. H. Jaffe, K. Fujii, M. Sengupta, H. Guerin and S. S. Epstein, *Life Sci.* **17**, 1051 (1968).
4. K. Fujii, H. Jaffe, Y. Bishop, E. Arnold, D. Mackintosh and S. S. Epstein, *Toxic. appl. Pharmac.* **16**, 482 (1970).
5. H. Jaffe, K. Fujii, H. Guerin, M. Sengupta and S. S. Epstein, *Biochem. Pharmac.* **18**, 1045 (1969).
6. R. Kato, A. Takanaka and H. Shogi, *Jap. J. Pharmac.* **19**, 315 (1969).
7. J. E. Casida, *A. Rev. Ent.* **8**, 39 (1953).
8. P. J. Creaven, D. V. Parke and R. T. Williams, *Biochem. J.* **96**, 879 (1965).
9. M. Klingenberg, *Archs Biochem. Biophys.* **75**, 376 (1958).
10. H. Wachs, *Science, N.Y.* **105**, 530 (1947).