

# The Inhibition of Beef Liver Hydrolytic Enzymes by Organophosphorus Pesticides —A Comparison of the Effects of Several Pesticides and Their Oxons on the Inhibition Response

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A procedure for determining the quantal inhibition characteristics ( $I_0^{50}$ ) of organophosphorus compounds that inhibit beef liver carboxylesterases has been described (1). As reported by other workers (2, 3) the oxygen analogs (i.e. oxons,  $P = O$ ) were all more potent esterase inhibitors than their parent compounds ( $P = S$ ).

The purpose of this study was to examine in more detail the effect of oxon formation on the inhibition response, and to compare the relative potencies of several structurally related pesticides.

## Materials

Organophosphorus Compounds. The four pesticides used in this study were: Co-Ral (0,0-diethyl 0-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl-phosphorothioate), Nemacide (0,0-diethyl 0-2,4-dichlorophenyl phosphorothioate), Ronnel (0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate) and Euelene (0-4-tert-butyl-2-chlorophenyl 0-methylphosphoramidate). The structural formulae for these compounds are shown in Figure 1.

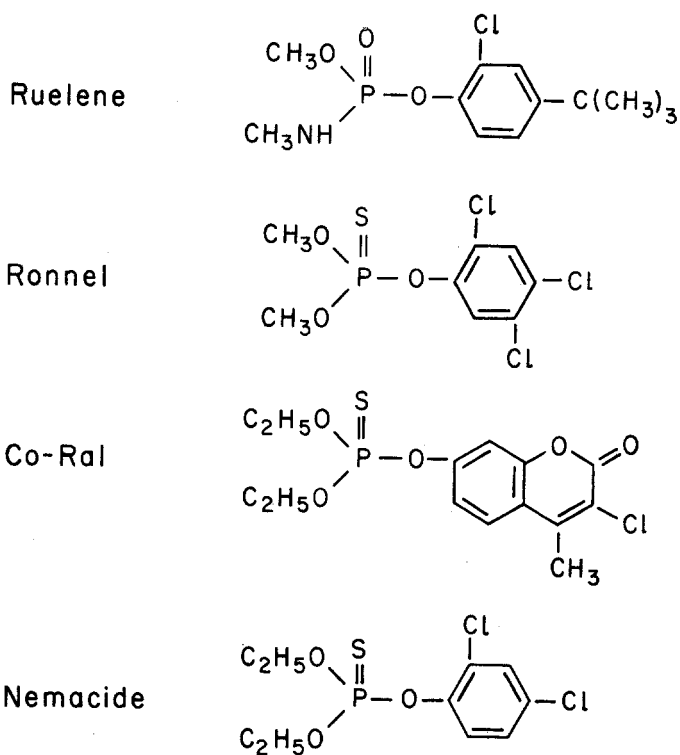


Figure 1. Organophosphorus Compounds Used in This Study.

The pesticides were obtained as analytical standards and made up in working solutions containing 10% methanol, a solvent chosen from previous experiments (4).

All other materials have been described previously (4).

### Procedure

The pesticide was preincubated with enzyme for 15 minutes at 25°C, the substrate added, and the reaction allowed to proceed for an additional 15 minutes at 25°C. The reaction was stopped by the addition of one drop DFP solution (this

compound is extremely toxic and should be handled with caution); the absorbance was read at 370 mμ (4) against a reagent blank, using a Beckman model B spectrophotometer, and compared to a control tube without pesticide. The inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Pesticide)}}{\text{Absorbance (Control)}} \times 100$$

The oxygen analogs were prepared from the parent compounds according to the method of Blinn (5), using m-chloroperbenzoic acid as oxidizing agent. Formation of the corresponding oxon (except for Ruelene which is already an oxon) was confirmed by I.R. spectroscopy and thin layer chromatography (6).

### Results and Discussion

The I.R. spectra of the four organophosphorus compounds before and after oxidation are shown in Figures 2-5

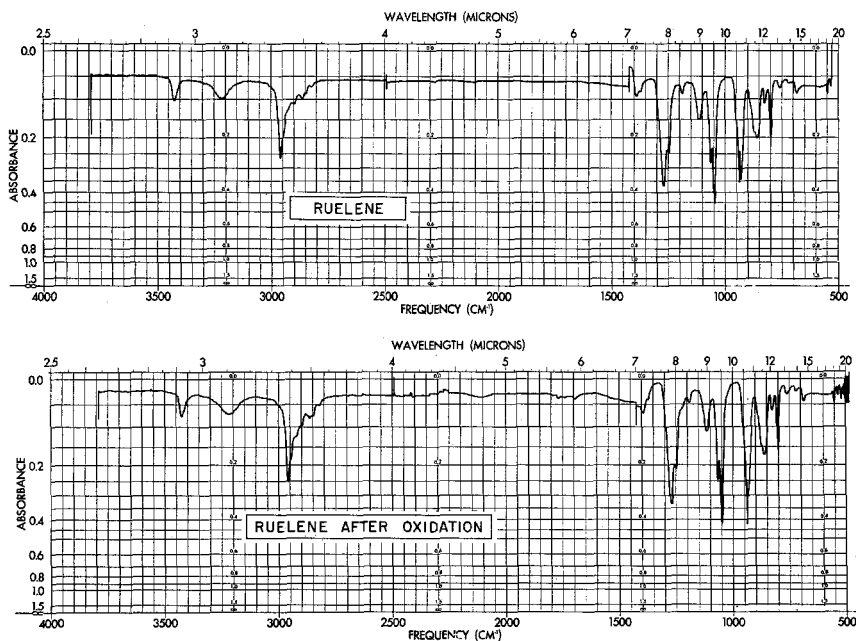


Figure 2. I.R. Spectra for Ruelene Before and After Oxidation.

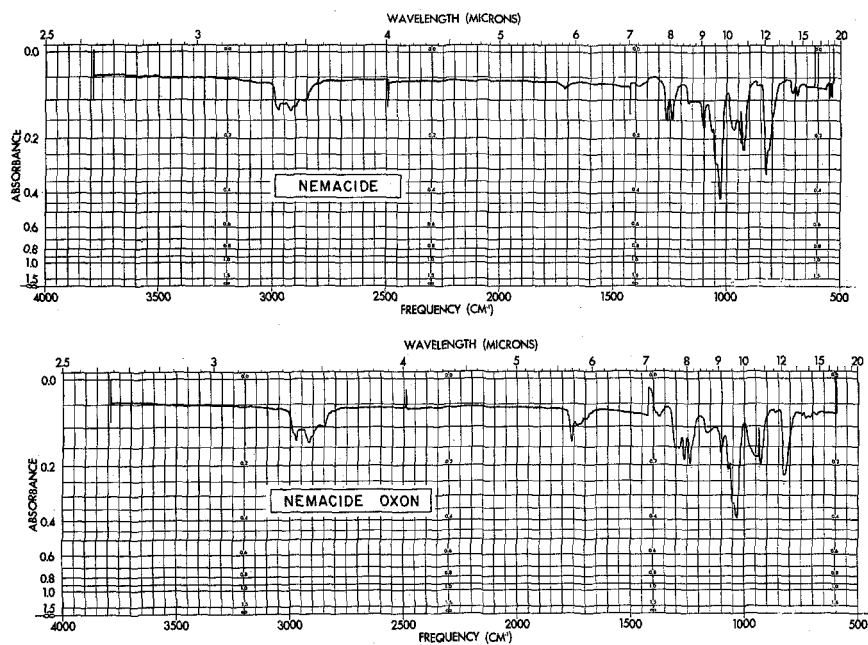


Figure 3. I.R. Spectra for Nemacide and Its Oxidation Product.

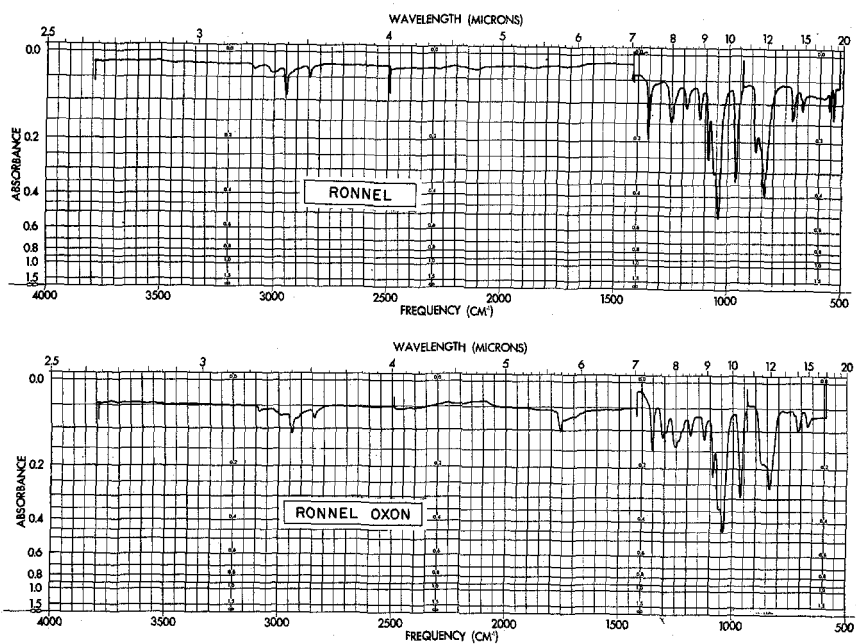


Figure 4. I.R. Spectra for Ronnel and Its Oxidation Product.

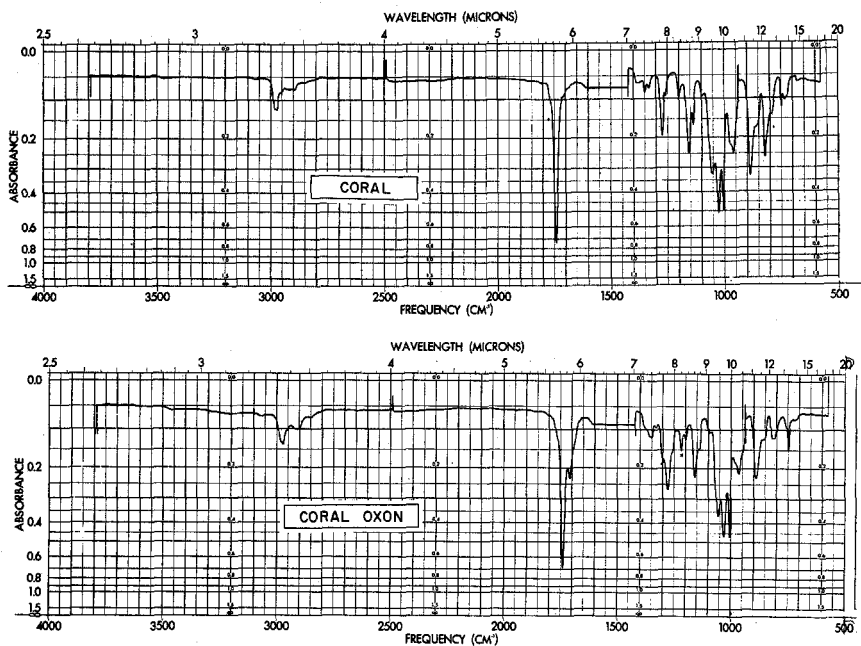


Figure 5. I.R. Spectra for Co-Ral and Its Oxidation Product.

The formation of the oxon seemed to coincide with the appearance of a new peak in the area of  $1300\text{ cm}^{-1}$ . Tracings of the thin layer chromatograms of the parent compounds and oxons are shown in Figures 6 and 7.

The chromatograms were run in 10% acetone hexane (v/v) and the plate was developed with 5-bromoindoxylacetate according to the method of Mendoza *et al.* (6). The first plate was not subjected to bromination (which converts the thiophosphate to the oxygen analog) and hence only the oxons appear. The second plate (Figure 7) was brominated prior to color development and all the compounds appear. The inhibition results are shown in Figure 8.

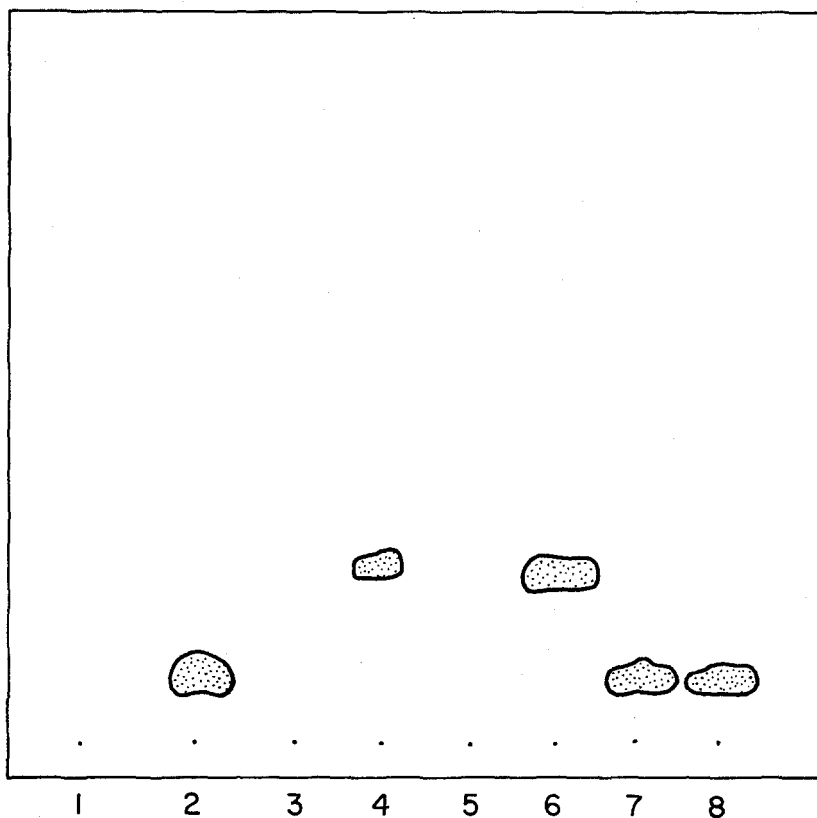


Figure 6. Thin Layer Chromatogram of Organophosphorus Compounds and Oxons before Bromination.  
1) Co-Ral 2) Co-Ral Oxon 3) Nemacide  
4) Nemacide Oxon 5) Ronnel 6) Ronnel  
Oxon 7) Ruelene 8) Ruelene after Oxidation.

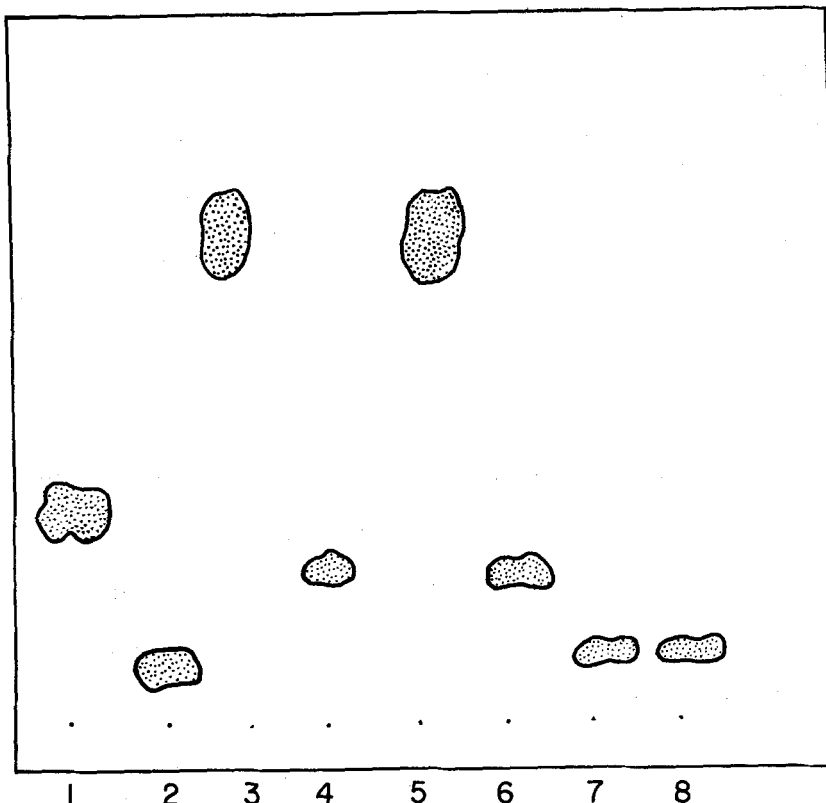


Figure 7. Thin Layer Chromatogram of Organophosphorus Compounds after Bromination. 1) Co-Ral 2) Co-Ral after Oxidation 3) Nemacide 4) Nemacide after Oxidation 5) Ronnel 6) Ronnel after Oxidation 7) Ruelene 8) Ruelene after Oxidation.

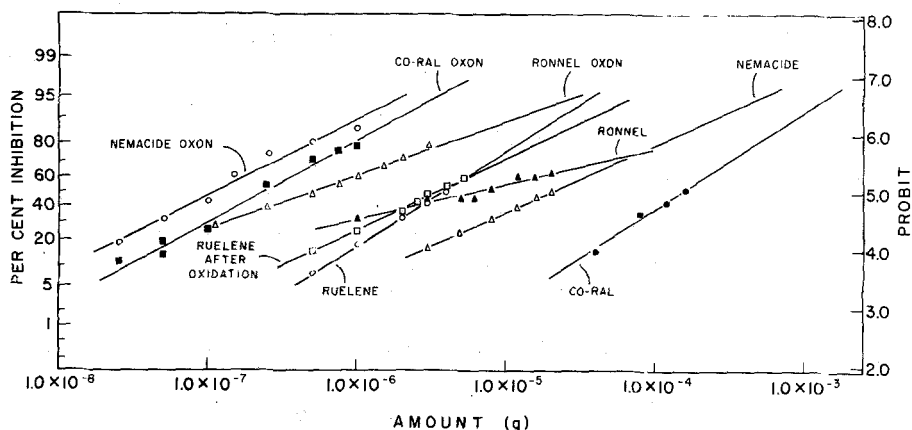


Figure 8. Inhibition Response Curves for Each of the Organophosphorus Compounds and Their Oxidation Products.

The response curve for Ruelene indicates that the oxidation procedure has had little effect on the compound's inhibitory properties. It also shows that the oxidation procedure does not produce any inhibitory by-products and that there is essentially a 100% recovery of the oxon from the oxidation step. The Ronnel oxon shows some increase in inhibition over the parent compound while the oxons of Nemacide and Co-Ral show a considerable increase over their parent compounds. The  $I_{50}$  values as well as a ratio representing the increase in inhibition for each of these compounds is shown in Table 1.

TABLE 1

Compound	Parent $I_{50}$ (gm/assay tube)	Oxon $I_{50}$ (gm/assay tube)	$\frac{\text{Parent } I_{50}}{\text{Oxon } I_{50}}$
Ruelene <sup>1</sup>	$3.7 \times 10^{-6}$	$3.4 \times 10^{-6}$	1.09
Ronnel	$5.8 \times 10^{-6}$	$5.0 \times 10^{-7}$	11.6
Nemacide	$2.0 \times 10^{-5}$	$1.05 \times 10^{-7}$	182.0
Co-Ral	$1.45 \times 10^{-4}$	$2.30 \times 10^{-7}$	630.5

<sup>1</sup> The two values given for Ruelene represent the inhibition before and after oxidation.

For Ruelene the parent/oxon ratio was 1.09, while for Ronnel this factor was 11.6, for Nemacide 182 and for Co-Ral 630.



The increased inhibitory properties due to oxon formation appear to be specific for the type of compound used. Where oxidation increases a compound's inhibitory potency and a new inhibition response curve is obtained, examination of these properties might provide a valuable means for the estimation and confirmation of organo-phosphorus pesticide residues.

#### Acknowledgements

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